

**BIOMPHALARIA SNAILS IN EAST AFRICA'S RIVER SYSTEMS: DISTRIBUTION,
GENETICS AND SCHISTOSOME INFECTIONS**

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DEDICATION

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ABBREVIATIONS AND ACRONYMS

μl: microlitre

ANOVA: Analysis of variance

Ca²⁺: Calcium ions

CCA: Circulating Cathodic Antigens

Cm: Centimetre

CO₂: Carbon dioxide

Df: Degrees of freedom

EPG: Eggs per Gram

FST: The Fixation Index

GLMM: Generalised linear mixed models

GIS: Geographic Information System

m: Metre

MDA: Mass drug administration

ml: Millilitre

mm : Millimetre

min.: Minute

MYA: Million Years Ago

pH: Measure of acidity or basicity

POC-CCA: Point of Care-Circulating Cathodic Antigen

WHO: World Health Organisation

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ABSTRACT

The population genetic structure of *Biomphalaria* snails was investigated. The degree of genetic variation and genetic population differentiation of *Biomphalaria* populations was inferred from the CO1 gene and the ITS-2 region. Phylogenetic relationships among the populations were deduced by PhyML version 2.4.4 using the Maximum Likelihood method, the GTR+G model, and with bootstrap values based on 1000 replicates. Measures of population differentiation were then calculated from CO1 sequence data and ITS-2 sequence data by use of the AMOVA model in Arlequin software version 3.5. The taxa identified were the '*B. pfeifferi* group' and the 'Nilotic Species Complex.' AMOVA revealed low genetic variation within *B. pfeifferi* populations (between 4.83% and 5.86%) and high genetic differentiation among the populations (between 94.14% and 95.17%). As for the 'Nilotic Species Complex' individuals, the genetic diversity within populations was moderate (between 28.78% and 72.17%) so was the genetic differentiation among populations (between 27.83% and 49.74%). There was a clear geographical clustering of haplotypes with the identification of 18 *B. pfeifferi* CO1 haplotypes and 20 CO1 haplotypes for the 'Nilotic Species Complex' individuals. As for ITS-2 sequence data, there was identification of 27 *B. pfeifferi* ITS-2 haplotypes and 23 haplotypes for the 'Nilotic Species Complex' individuals.

An extensive study on the distribution of *Biomphalaria* snails in East Africa's River systems was conducted. Malacological surveys were conducted at a total of 172 sites with ecological and physicochemical parameters associated with snail abundance recorded and the CO1 gene marker used to differentiate the *Biomphalaria* snails to their respective species groups. *Biomphalaria* snails were found in a total of 31 out of the 172 visited sites with *B. pfeifferi* populations being found at 23 sites and members of the 'Nilotic Species Complex' being found at 9 sites. Streams proved to be the habitats most preferred by the *Biomphalaria* snails (61.3% of all the sites where the snails were found were streams). Regarding ecological and physicochemical parameters, temperature and water depth proved to be the only factors that had a statistically significant positive relationship with the abundance of the snails. There was also molecular detection of *S. mansoni* infections in *Biomphalaria* snails using ND5 and SMF/R molecular makers. Schistosome infection was detected at 11 out of the 31 sites where *Biomphalaria* snails were found. We observed low to moderate incidences of *S. mansoni* infection with an overall infection rate in infected sites of 21.27%. Out of the 11 populations that were found infected with *S. mansoni*, all of them were *B. pfeifferi* populations; none were 'Nilotic Species Complex' populations.

Furthermore, the population genetic structure of pre-patent and sub-patent *S. mansoni* populations was examined with the use of the ND5 mitochondrial gene. Moderate levels of genetic diversity were found within (53.34%) and between (46.66%) the *S. mansoni* populations. Based on the findings presented in this thesis, future work on *Biomphalaria* snails in East Africa's River systems can include conducting cercarial shedding studies on the snails from sites where the snails were found and use of molluscicides as part of an integrated approach for schistosomiasis control in locations where *Biomphalaria* snails were found.

CHAPTER ONE: GENERAL INTRODUCTION

This chapter introduces Schistosomiasis, *Schistosoma* parasites, snail intermediate hosts and places them in the context of transmission of schistosomiasis across the globe. The current state of the disease, the effect of climate change on future transmission of the disease and the significance of snail intermediate hosts as far as schistosomiasis is concerned are discussed in the chapter. This overview sets the prelude and leads to the introduction of the main research questions of this PhD thesis.

1.1 SCHISTOSOMIASIS

Schistosomiasis is a parasitic disease caused by flatworms that belong to Phylum Platyhelminthes, Class Trematoda, Family *Schistosomatidae* and Genus *Schistosoma*. Schistosomiasis was first described by a German physician known as Theodore Bilharz in the mid 1800's during an autopsy procedure in Egypt (Bilharz, 1853). Fossil records indicate that *Schistosoma* parasites have been in existence for thousands of years. *S. haematobium* eggs have been found in Egyptian mummies, dating more than 6,000 years ago (Bella *et al.*, 2018). There is also archaeological evidence that suggests that the evolution of *Schistosoma* parasites and the evolution of human beings are closely associated (Anastasiou *et al.*, 2014). Furthermore, descriptions fitting acute schistosomiasis (also known as Katayama syndrome) have been found in Chinese medicine literature dating 2,400 years ago (Stefano *et al.*, 2018). This evidence suggests that schistosomes have been in existence for almost the same time that human beings have been in existence. Human beings have been in existence for 4-8 million years (Ruvolo, 1997).

Schistosome infections are debilitating and affect more than 1 billion people worldwide (WHO, 2013). It is estimated that at any given time about 250 million people are always infected with schistosomiasis. According to the World Health Organisation, more than 200,000 deaths that occur annually are attributed to the disease (WHO, 2013). Schistosomiasis is ranked second to malaria in terms of diseases of public health importance in the tropics and subtropics (Gryseels, 2012). Public health importance in this context means morbidity and mortality associated with a disease. Most cases of schistosomiasis are found in Africa (80%-90% of cases), followed by Asia and then South America (Figure 1). Cases of schistosomiasis have been reported in 78 countries of the world (Gryseels, 2012). In East Africa, it is estimated that about 40 million people are infected with schistosomiasis and about 140 million people are at risk of infection (Aula *et al.*, 2021). Schistosome infections havearte debilitating effects on infected individuals; they render them unproductive due to the disability and malaise that result from the infections (Gryseels, 2012). According to WHO, (2013), the debilitating effects of schistosomiasis impair close to 4 million individuals yearly from engaging in any productive economic activity. *Schistosoma* parasites not only affect a person's physical wellbeing but also their neuro-cognitive state. Neurological complications associated with schistosomiasis have been reported and these are normally orchestrated by deposition of schistosome eggs in the spinal cord and brain of infected individuals. Neurological symptoms associated with schistosome infection include loss of consciousness, increased headache, increased nausea, delirium, dysphasia, focal motor deficits and visual field impairment (Carod, 2008). Schistosomiasis, urogenital schistosomiasis to be specific has been revealed as a risk

factor to HIV (Human Immunodeficiency Virus) and a potential enhancer of HIV disease progression (Mbabazi *et al.*, 2011).

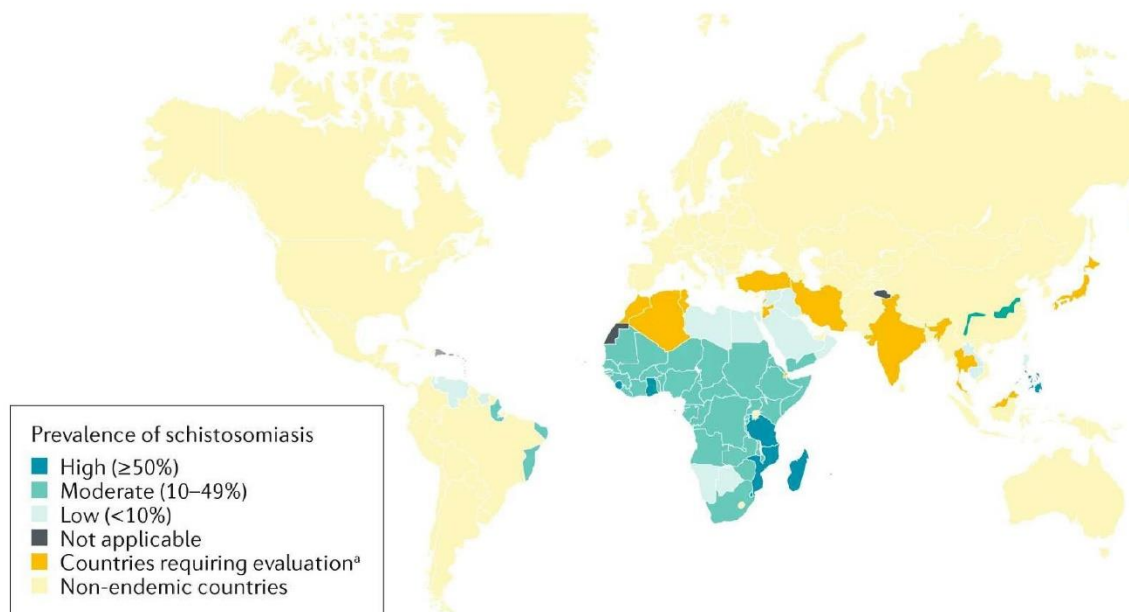


Figure 1: Global distribution of schistosomiasis (WHO, 2013).

Nigeria has the highest incidence and prevalence of schistosomiasis in the world (29 million cases) with cases of both intestinal and urogenital schistosomiasis being reported in the country (Figure 2). Tanzania has the second highest incidence of schistosomiasis in Africa, (19 million cases) with the entire country being at risk of being infected with the disease. The Democratic Republic of Congo and Ghana tie at number three in respect to the prevalence of schistosomiasis in Africa, with 15 million reported cases (Adenowo *et al.*, 2015).

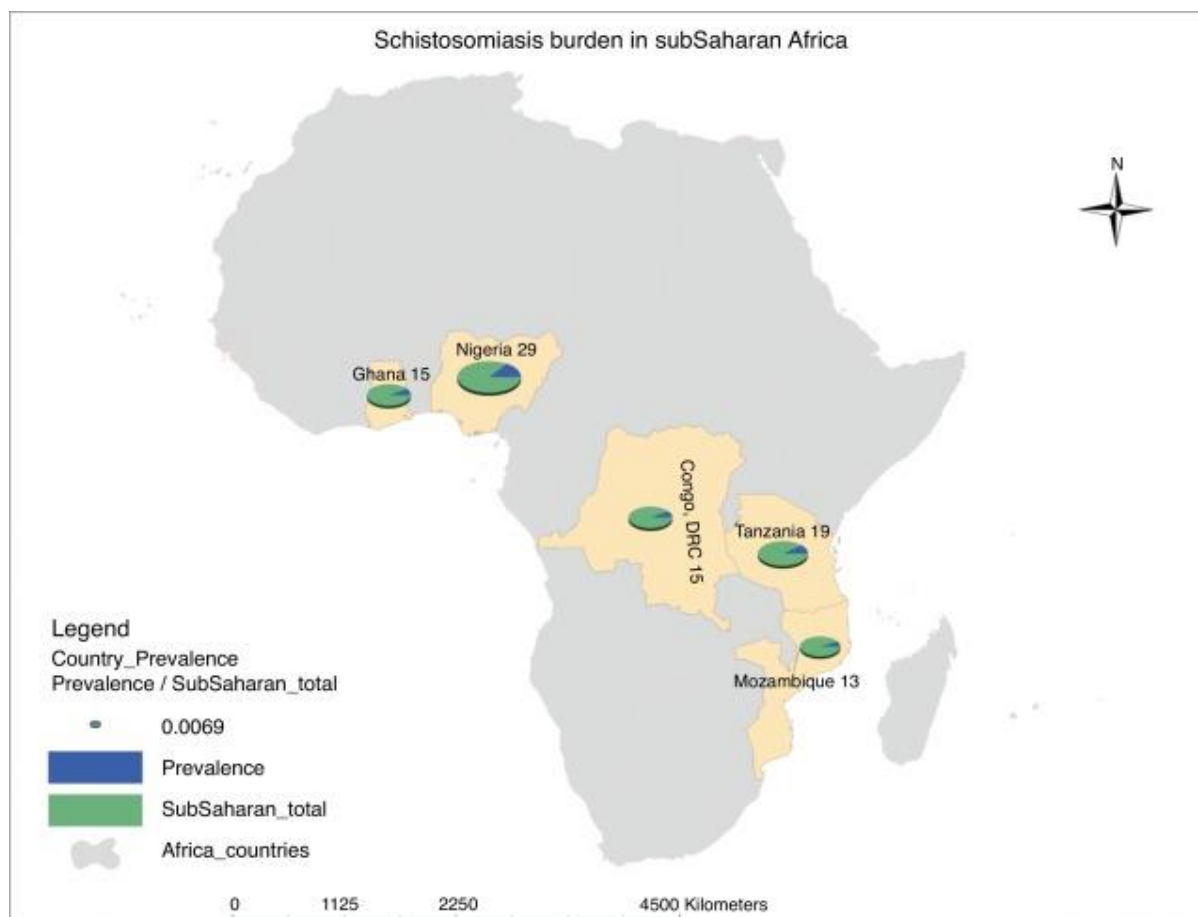


Figure 2: Schistosomiasis burden in sub-Saharan Africa, courtesy of Adenowo *et al.* (2015).

Schistosomiasis causes approximately 3.5 million disability adjusted life years (DALYs) (Tchuem *et al.*, 2017). DALYs refers to the total number of wasted years due to the inability to work because of malaise and physical disability that stem from schistosome infections. However, the significance of schistosomiasis from a public health perspective keeps on being overlooked in countries where the disease is endemic; that's the reason the disease is classified as a neglected tropical disease (Gryseels, 2012).

Schistosomiasis is regarded by the World Health Organisation as a disease of the poor because schistosome infections and other related diseases like soil transmitted helminthiases are often linked to poor environmental hygiene, poverty, poor health infrastructure and poor personal hygiene. Transmission of schistosomiasis commences when people urinate or defecate in water bodies and pass out *Schistosoma* eggs. The behaviour of urinating and defecating is partly because of ignorance and because of lack of public and home latrines (WHO, 2013). Recently, cases of schistosomiasis have been reported in Europe; partly attributed to climate change and movement of illegal immigrants from endemic African countries to Europe (Cobo, 2014).

A study conducted by Clements *et al.* (2010) in East African Great Lakes Region is one of the most extensive studies conducted in East Africa to establish *S. mansoni* infection rates in human beings. In the study, other than ascertaining *S. mansoni* infection rates, the

prevalence of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworms (*Ancylostoma duodenale* and *Necator americanus*) were also established (Figure 3).

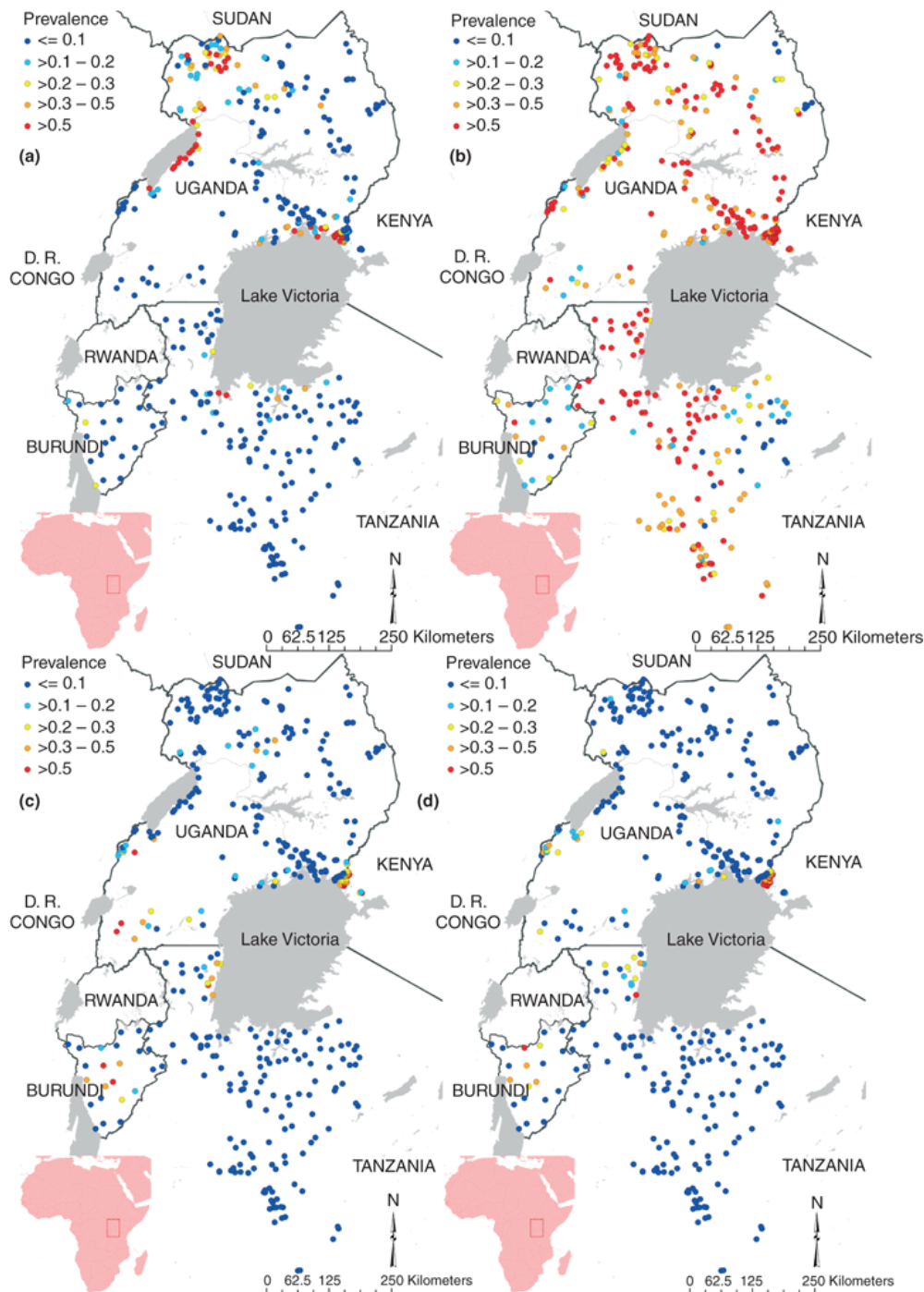


Figure 3: Prevalence of (a) *Schistosoma mansoni*, (b) hookworm; (c) *Ascaris lumbricoides* and (d) *Trichuris trichiura* in school-aged children, Great Lakes region, East Africa 1998–2005; courtesy of Clements *et al.* (2010).

1.1.1 *Schistosoma* parasites

Schistosomes are of biomedical importance because they are parasites of vertebrates, including humans. The genus *Schistosoma* consists of 22 species that are described based on the morphology of their eggs as well as the intermediate host that they exploit (Littlewood & Bray, 2001). *Schistosoma* parasites have no anus, they cannot excrete their waste and because of that, they regurgitate their waste back into their hosts bloodstream (Colley *et al.*, 2014). Definitive hosts for *Schistosoma* parasites are mammals (where development of sexual stages of the parasite takes place) and they include man and cattle. Intermediate hosts (where development of asexual stages of the parasite takes place) are aquatic snails. There are also occasions where *Schistosoma* parasites rely on reservoir hosts for survival and continuity, mostly when they face extinction (Colley *et al.*, 2014). These include rodents and wildlife such as vervets, rats, chimpanzees, and baboons (Gryseels, 2012; Bakuza *et al.* 2017). In the 1950's, *S. mansoni* and *S. haematobium* were found in many rodents from the Democratic Republic of Congo. *S. mansoni* have also been found in rodent populations from South Africa, Senegal, Egypt, and Sudan. In recent years, *S. mansoni* have been found in rodent populations from Western Kenya (Standley *et al.*, 2012b). Human to wildlife interactions play a major role in transmission of *Schistosoma* parasites from reservoir hosts to humans. Shrinking of wildlife habitats has contributed a great deal in bringing human beings and wildlife close together, subsequently leading to an increased risk of humans and wildlife interacting and sharing diseases. Habituation in game parks and animal conservancies in a way plays a role in increasing transmission of pathogens, parasites and diseases between wildlife and humans. Parasites that infect humans are almost similar to those that infect non-humans, more so in sympatric areas (Bakuza *et al.*, 2017).

Schistosoma parasites as parasites of humans, non-human primates, rodents, wild-artiodactyls, domestic artiodactyls, perissodactyls, insectivores and carnivores are widely distributed across, straddling East Asia, South East Asia, Africa, West, South Asia and the Neotropics (Figure 4).

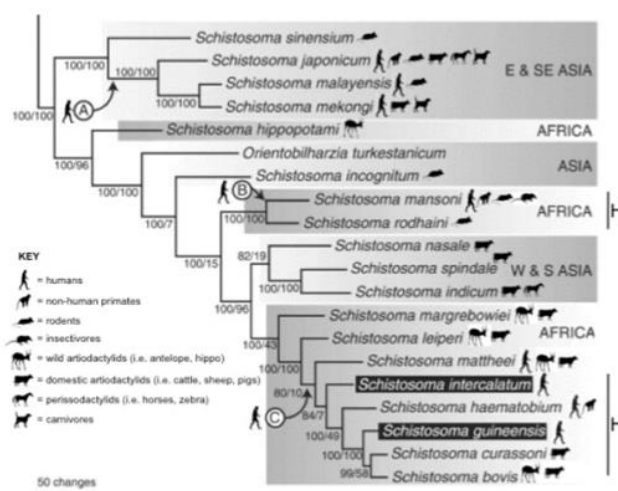


Figure 4: An image showing currently recognized *Schistosoma* species with their respective definitive hosts and geographical range, courtesy of Standley *et al.* (2012b).

1.1.2 Origin of *Schistosoma* parasites

The origin of *Schistosoma* parasites is a debate that has been going on for several years. There is a theory that suggests that the progenitor of *Schistosoma* parasites originated from Asia and travelled to Africa from where *Schistosoma* parasites spread to the Neotropics. There is also a theory that suggests *Schistosoma* parasites originated from Africa and spread to Asia, the Middle East, and the Neotropics from Africa (Lawton *et al.*, 2011).

According to the theory that proposes *Schistosoma* parasites originated from Asia, the main argument is that the most basal clade in genus *Schistosoma* is of Asian descent with *S. japonicum* rooted at the base (Lockyer *et al.*, 2003). As for *S. mansoni* it is stated to be a derivative of an early schistosome that colonised Africa from Asia with the radiation process taking place in East Africa (Morgan *et al.*, 2005). The theory is further supported by evidence that the *S. mansoni* species in East Africa exhibits a significant amount of genetic variation compared to *S. mansoni* species found in other locations of the world. Morgan *et al.* (2005) avers that *S. mansoni* in East Africa comprises of three clades and a few basal members of a possible fourth clade. Morgan *et al.* (2005) further asserts that the mitochondrial genome of *S. mansoni* populations from East Africa show a high level of mitochondrial differentiation when compared to that of *S. mansoni* populations from other regions of the world. In addition, *S. mansoni* found in South America have a high affinity to *S. mansoni* found in West Africa, suggesting that the transatlantic trade and movement played a role in the colonisation of South America by *S. mansoni* (Morgan *et al.*, 2005).

According to the theory that proposes *Schistosoma* parasites originated from Africa, it is stated that the genus *Schistosoma* arose in Africa 150 million years ago (MYA) before the separation of the supercontinent Gondwanaland (which constituted present day Africa, South America, Antarctica, and Australia) and that the spread to other parts of the world was because of continental drift. The ancestor of the Asian *Schistosoma* parasites was later carried to Asia through India as Asia moved away from Africa where radiation arose, giving rise to *S. japonicum* and *S. indicum*. It is further argued that the stock of *Schistosoma* parasites that remained in Africa after the separation of Gondwanaland radiated, giving rise to *S. mansoni* group and *S. haematobium* group species members. From Africa, *S. mansoni* was able to migrate to the Neotropics (Davis *et al.*, 1993).

1.1.4 Classification of Schistosomiasis

Classification of schistosomiasis is generally done based on the nature of the definitive host. The three broad categories of schistosomiasis are livestock schistosomiasis, avian schistosomiasis, and human schistosomiasis.

Livestock schistosomiasis refers to a form of schistosomiasis where livestock act as the definitive hosts with the sexual development of schistosomes taking place in a livestock host. Livestock such as horses, goats, pigs, camels, cattle, and sheep are susceptible to schistosome infections. *Schistosoma* species that affect livestock include *Schistosoma bovis*, *S. hippopotami*, *S. guineensis*, *S. indicum*, *S. curassoni*, *S. mattheei*, *S. rodhaini*, *S. nasalis*, *S. margrebowiei*, *S. leiperi*, *S. spindale*, *S. kisumusensis* and *S. intercalatum*. Livestock *Schistosoma* species belong to what is generally known as the non - human

Schistosoma haematobium group (Hanelt *et al.* 2009; Webster *et al.* 2006). *S. bovis* and *S. mattheei* are primarily parasites of cattle. However, *S. bovis* and *S. mattheei* have also been found in rodents, baboons, horses, and zebras. *S. curassoni* is primarily found in cattle, sheep, and goats. *S. leiperi* and *S. margrebowiei* have been found in antelopes, zebras, and horses. *S. kisumuensis* has been found in rodents and small insectivores. *S. hippopotami* has been found in hippopotamus whereas on the other hand, *S. rodhaini* has been found in rodents.

Avian schistosomiasis refers to a form of schistosomiasis where birds are the definitive hosts and with the sexual development of schistosomes taking place in a bird host. Avian schistosomes constitute the largest clade of Family *Schistosomatidae*. The clade has a total of 10 genera. The largest genus of parasites that cause avian schistosomiasis are called *Trichobilharzia* (Ahmed *et al.*, 2020). Members of genus *Trichobilharzia* infect more than 40 bird species. Avian schistosomes have been detected in mesenteric veins, portal veins and testicular veins of several birds. However, avian schistosomiasis is most prevalent in waterfowl birds. Other avian schistosomes belong to genera *Ornithobilharzia*, *Allobilharzia*, *Jilobilharzia*, *Bilharziella*, *Macrobilharzia*, *Dendrobilharzia*, *Gigantobilharzia*, *Austrobilharzia* and *Anserobilharzia*. Snail intermediate hosts for avian schistosomes are freshwater snails. Avian schistosomes have zoonotic potential but there are no current reports of avian schistosomes infecting human beings (Ahmed *et al.*, 2020). However, cercariae of avian schistosomes are regarded as the greatest causative agents of human cercarial dermatitis, also known as swimmer's itch. Human cercarial dermatitis is a skin condition that affects people globally courtesy of recreational and occupational reasons in freshwater areas. In addition, avian schistosomes have been detected in rodents and monkeys (Elham *et al.*, 2020).

Human schistosomiasis refers to a form of schistosomiasis where humans are the definitive hosts, with the sexual development of schistosomes taking place in a human host. Human schistosomiasis is further classified into two divisions, intestinal schistosomiasis, and urogenital schistosomiasis (Gryseels, 2012). Intestinal schistosomiasis is a type of schistosomiasis where the adult schistosomes reside in the mesenteric veins of the definitive host. Urogenital schistosomiasis on the other hand is a form of schistosomiasis where the adult schistosomes reside in the veins that drain the main pelvic organs which include cervix, uterus, and the urinary bladder (venous plexous veins). In recent years, it has been discovered that urogenital schistosomiasis is related to squamous cell carcinoma of the urinary bladder well as well as female genital schistosomiasis, with the latter highly associated with a higher risk to HIV and infertility (Santos *et al.*, 2021).

Human intestinal schistosomiasis is caused by *Schistosoma mansoni*, *Schistosoma japonicum*, *Schistosoma mekongi*, *Schistosoma guineensis* and *Schistosoma intercalatum*. Urogenital schistosomiasis on the other hand is caused by *Schistosoma haematobium* (WHO, 2013). *S. mansoni* and *S. haematobium* are responsible for majority of the human schistosome infections in Africa, Middle East, and South America. *S. mansoni* on its own is responsible for more than 83 million schistosome infections in the world (Morgan *et al.*, 2005). In addition, *S. mansoni* infections are prevalent in more than 54 countries of the world, predominantly Africa. *S. mansoni* is endemic in Africa, South America, Central

America, and the Middle East (Morgan *et al.*, 2005). An Asian schistosome species responsible for intestinal schistosomiasis, *S. mekongi* is endemic in countries along which the Mekong River flows including Laos, Cambodia, and Thailand (Zhou *et al.*, 2014). *S. japonicum* is primarily found in the Philippines, Indonesia and China and its infections have been reported in many domestic and wild animals' species (including buffaloes), with some of the animals playing an important role, serving as reservoir hosts for the parasite. *S. japonicum* is slightly different from other human *Schistosoma* parasites because other than infecting humans it also infects animals such as pigs, domestic dogs, buffaloes, and cattle. *S. japonicum* is regarded as the only zoonotic species of genus *Schistosoma*. Eggs of *S. japonicum* have been found in faeces of dogs, human beings, cats, buffaloes and other mammalian species, totalling at least forty in number). An African *Schistosoma* species, albeit with little public health significance, *S. intercalatum*, has a limited geographical range to parts of Central Africa and West Africa. *S. guineensis* is another *Schistosoma* parasite that infects man but with little public health significance (it was first described in 2003 and has been found in a few parts of West Africa, specifically in the Lower Guinea region). *S. mekongi*, *S. guineensis* and *S. intercalatum* are less significant from an epidemiological perspective because they are less widespread (Gryseels, 2012). It is only three *Schistosoma* species that have been implicated as being of public health importance and these are *S. mansoni*, *S. haematobium* and *S. japonicum* (Standley *et al.*, 2012b).

Schistosoma parasites are atypical trematodes; typical trematodes are hermaphrodites, whereas *Schistosoma* parasites are dioecious with male and female worms living separately except when they come together to mate. Male *Schistosoma* parasites are flat whereas female *Schistosoma* parasites are cylindrical (Secor & Colley, (2005). *Schistosoma* species that cause human schistosomiasis in Africa are *S. mansoni*, *S. haematobium*, *S. intercalatum* and *S. guineensis* (Figure 5).

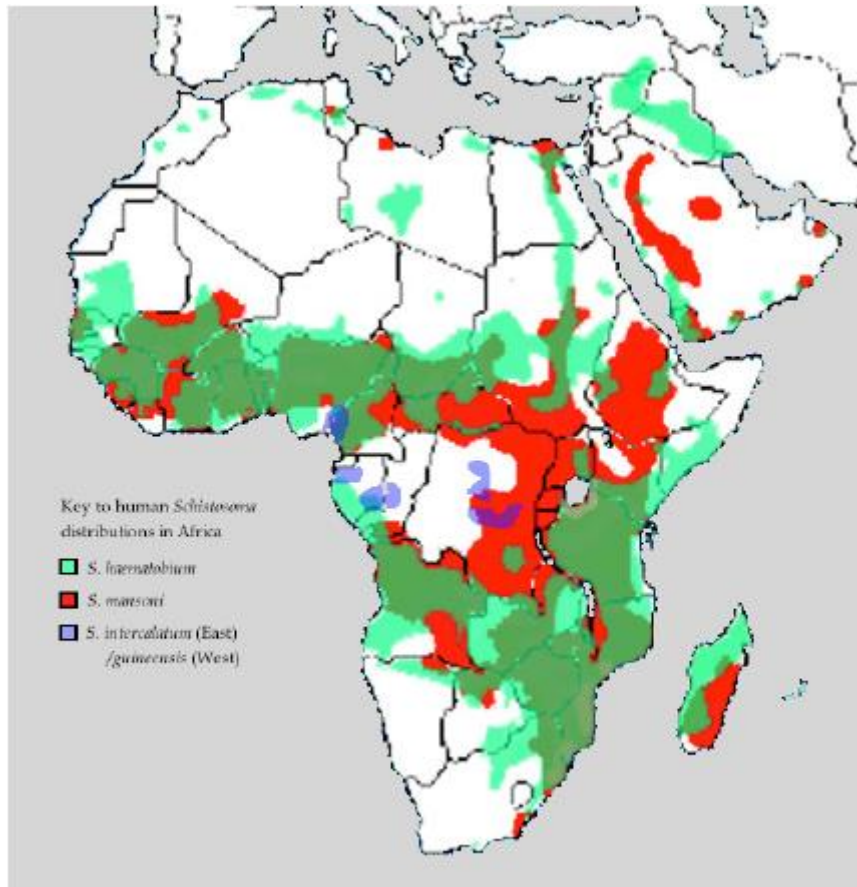


Figure 5: The distribution of *Schistosoma* species in Africa, courtesy of Standley *et al.* (2012b).

The egg morphology of the different human *Schistosoma* parasites can be used to identify them to the species level. *S. mansoni* eggs have a lateral spine that is conspicuous. On the other hand, eggs of *S. japonicum* are round like *S. mekongi* eggs, however eggs of *S. mekongi* have a terminal spine that is small in size and inconspicuous. Lastly, *S. intercalatum* eggs exhibit a bulging characteristic at the centre together with a terminal spine (Figure 6).

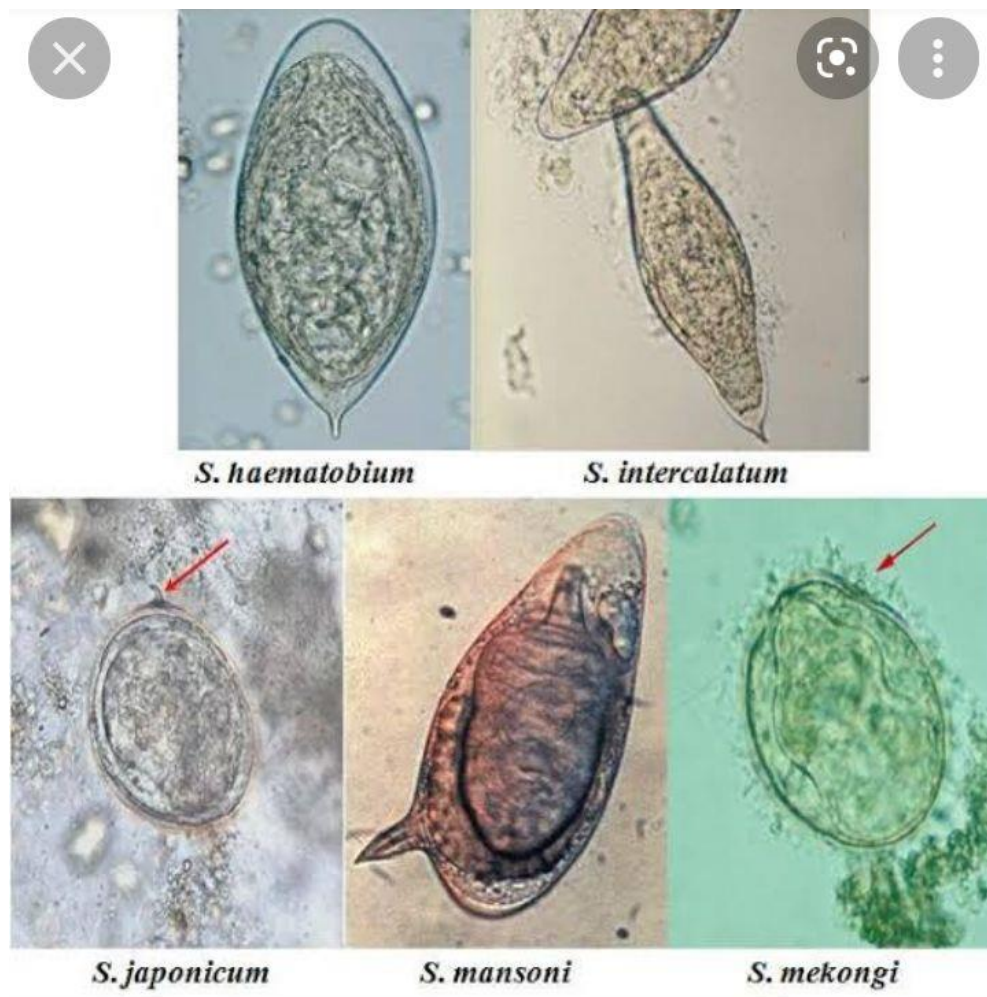


Figure 6: Image of *Schistosoma* parasites eggs courtesy of Bhagyashree (2010).

1.1.3 Schistosome hybrids

Schistosome hybrids refer to a mixed breed of two different schistosome species. When two different schistosome species genetically interact, schistosome hybrids are formed. All the current schistosome hybrids that have been discovered in nature, involve *S. haematobium* lineages. It is important to note that *S. haematobium* naturally infects non-human primates and that the hybrids are formed courtesy of introgression, a type of gene flow that involves two populations of different species (genetic material is transferred from one species to the gene pool of a different species) (Webster *et al.*, 2019). There is a growing interest on Schistosome hybrids because in the past, no one thought such an arrangement would be possible and also because the hybrids are now changing perspectives on how to approach schistosomiasis control. Schistosome hybrids have the potential of altering dynamics on schistosomiasis control and prevention approaches. It has been revealed that Schistosome hybrids are more infectious compared to single species schistosomes and that they pose a high risk of zoonotic transmission (Webster *et al.*, 2019). Additionally, the hybrids pose a threat to current control initiatives because current eradication programmes are tailored to address only human schistosomes and not human-livestock schistosome hybrids. Furthermore, because human-livestock schistosome hybrids have a heightened level of egg production and the ability to utilise a wider range of

snail intermediate hosts, this means that they can easily expand their geographical range as far as transmission of schistosomiasis is concerned.

Schistosome hybrids are generally associated with zoonosis (Kincaid-Smith *et al.*, 2017). Natural hybrids of *S. haematobium* - *S. bovis* have been reported among children living next to the Senegal River basin (Standley *et al.*, 2012b). In recent years, *S. bovis* has received a lot of attention due to the emergence of the *S. haematobium* - *S. bovis* hybrid, which has a higher virulence, a higher prevalence rate, a higher level of compatibility with different snail intermediate hosts and a geographically widespread sympatric transmission (Kincaid-Smith *et al.*, 2017). *S. haematobium* - *S. bovis* hybrids were first detected in cattle in Benin, West Africa (Savassi *et al.*, 2020). In snail intermediate hosts, the hybrids have been found in Senegal and Corsica dwelling in *Bulinus truncatus* snails (Kincaid-Smith *et al.*, 2017). *S. haematobium* and *S. bovis* differ in their tropisms with *S. haematobium* causing urogenital schistosomiasis whereas *S. bovis* causes mesenteric schistosomiasis (Olivier *et al.*, 2021).

Recent studies around Lake Malawi, have revealed the existence of a *S. haematobium* - *S. mattheei* hybrid complex (Figure 7). The development of the *S. haematobium* - *S. mattheei* hybrid complex could be attributed to the fact that *Bulinus* snails (mostly *B. globosus*) serve as the snail intermediate host and the complex was likely formed when miracidia from the two *Schistosoma* species fused in the *Bulinus* snail, forming the hybrid complex (Stothard *et al.*, 2020). *S. haematobium* - *S. mattheei* hybrid complex is associated with increased egg laying and high disease potentials as it is for the case of *S. haematobium* - *S. bovis* hybrid. Schistosome hybrids are likely to jeopardise preventive chemotherapy efforts because mass drug administration programmes only target humans, not livestock and given that the lifecycle of the hybrids involves both livestock and humans, this makes control measures through chemotherapy self-defeating (Olivier *et al.*, 2021). Hybridisation is also likely to increase the colonisation potential of schistosomiasis, creating new transmission foci (Boissier *et al.*, 2016).

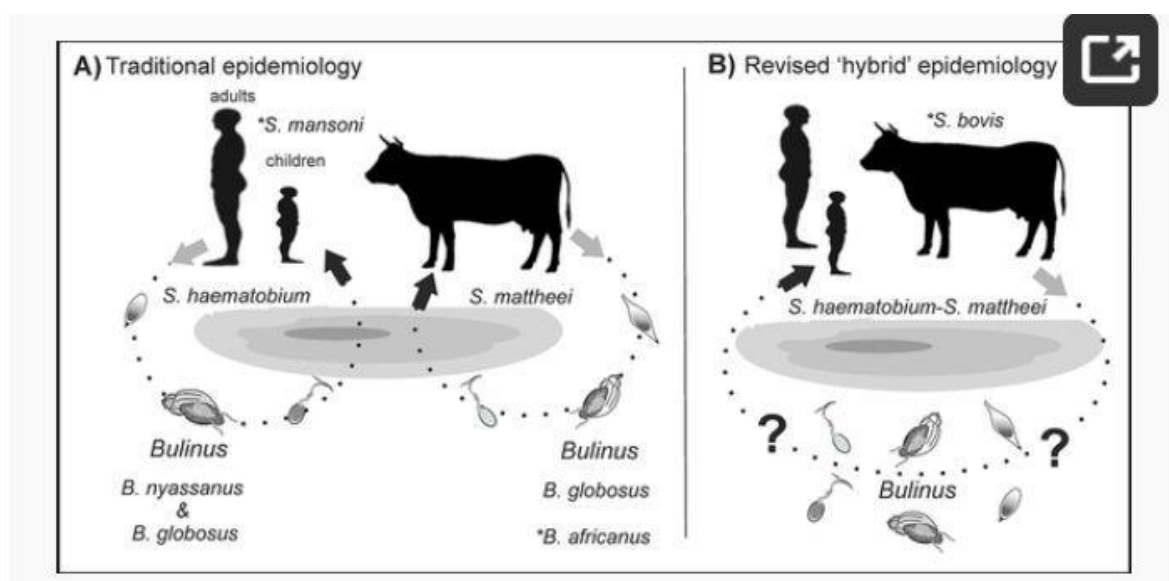


Figure 7: An image showing traditional epidemiology of *S. haematobium* and 'hybrid' epidemiology of *S. haematobium*, courtesy of Stothard *et al.* (2020).

There are reports indicative of an *S. mansoni*-*S. rodhaini* hybrid. Reports on the *S. mansoni*-*S. rodhaini* hybrid assert that the hybridization naturally occurs in rodents, snail intermediate hosts but not in mammals. It has also been revealed that the *S. mansoni*-*S. rodhaini* hybrid has the potential of being zoonotic, but there has been no confirmation of such an occurrence yet (Standley *et al.*, 2012b). *Schistosoma intercalatum/guineensis* hybrids with either *S. haematobium* or *S. bovis* have also been reported in areas where there is an overlap in the geographical range of the four species as well as the presence of ideal snail intermediate hosts (Standley *et al.*, 2012b).

Zoonotic transmission of parasitic infections from human beings to non-human primates has been reported in a few national parks and forest reserve areas in Africa. These include Gombe National Park and Mahale Mountains National Park, both found in the United Republic of Tanzania. Given that zoonotic transmission of infections has been reported in areas that one would expect to be highly protected, with little human to animal interaction, it means that the chances of schistosome hybrid transmissions occurring in non-protected areas are very high (Standley *et al.*, 2012b). Courtesy of ultrasound imagery, significant liver pathology in chimpanzees, a characteristic of chronic schistosomiasis was revealed, affirming the potential of zoonotic transmission of schistosome infections between human beings and non-humane primates (Standley *et al.*, 2012b).

1.1.4 Factors associated with endemicity of human schistosomiasis

There are several factors that contribute to human schistosomiasis being more prevalent in one locality than the other. These factors can generally be classified into six categories: poverty, expansion of water projects, lack of political will, age, gender, and occupation (Afifi *et al.* 2016; Bernin & Loter, 2014; Zhou *et al.* 2014; Muhumuza *et al.* 2009).

Poverty is a major reason schistosomiasis continues to be a public health problem in the world. Individuals engrossed in poverty have no choice but to continue to live lifestyles that put them at risk of being infected with *Schistosoma* parasites. Lack of clean water supplies and inability to access latrines among poor folk makes them highly susceptible to schistosome infections. In poor rural areas there are no proper sanitation facilities. Individuals who live in such low resource settings are also illiterate and semi-illiterate hence not well informed about proper public health practices (Savioli *et al.*, 2002). Most human populations along the Mekong River depend on the river for washing, bathing, swimming, and fishing. This explains how important the river is to their livelihoods, meaning that they have little to no choice as far as coming in contact with the waters in the river is concerned (Zhou *et al.*, 2014). Little family income dissuades families in areas where schistosomiasis is endemic from constructing proper latrines that would play an important role in thwarting the life cycle of *Schistosoma* parasites (Muhumuza *et al.*, 2009). This is because those families have more pressing needs, food to be specific being the first priority. Construction of pit latrines in some poor localities is viewed as an 'expensive' venture, leaving people in such communities to defecate and urinate in the open (Muhumuza *et al.*, 2009). Low literacy levels associated with poverty is also linked to high schistosome infection rates. Individuals with low literacy levels lack the necessary public health knowledge that would ensure that they take the necessary precautions that would prevent them from being infected by the parasites (Okanla *et al.*, 2003).

Development and expansion of water projects in tropical and sub-tropical countries has contributed to an increase in cases of schistosomiasis in those countries. Schistosomiasis is a water borne disease and extensive water projects provide an ample breeding ground for snail intermediate hosts. Transmission of schistosomiasis only takes place in areas where there is existence of snail intermediate hosts and where there is frequent contact of humans with schistosome infested waters (Sturrock, 1965). Irrigation rice farming in Africa has played a major role in increasing the transmission of schistosomiasis (Boelee & Madsen, 2006). Cases of intestinal schistosomiasis have been found to be more prevalent in areas where rice is grown in swamp fields than where it is grown in upland areas (Boelee & Madsen, 2006). However, reports show that swamp rice farming is more productive than upland rice farming, leading to an increase in swamp rice farming in Africa (Boelee & Madsen, 2006). In swamp rice farming, original vegetation is removed followed by the construction of field depressions that are later filled with water. Water canals are then constructed, water pumped into the canals and then from the canals, water is directed into the field depressions. Rice is then grown into the flooded depressions (Boelee & Madsen, 2006).

Lack of political will to fight against schistosomiasis, more so in highly endemic countries continues to be a hindrance in the fight against schistosomiasis. Infected people and those that are at risk of being infected lack better representation in most legislative platforms as far as decision making in most jurisdictions is concerned leading to failure in creation of better policies to prevent, control and eliminate the disease (Colley *et al.*, 2014).

Age and gender are important demographic factors in the transmission of schistosomiasis. Women, young men, and boys are more prone to being infected by schistosomes, due to the fact that they engage more in domestic, economic and recreational activities in water bodies, more so in rural settings (Boelee & Madsen, 2006). Schistosome infections are more prevalent among school aged children and more so in teenagers (Omer & Abdel, 1976). According to Afifi *et al.* (2016), schistosome infections often peak between 10 to 20 years of age. This is attributed to the fact that individuals who belong to this age group engage in more water related activities such as swimming, fishing, and washing than any other age group. A study conducted in Brazil by Lima *et al.* (1991) revealed that infections and re-infections among individuals who are less than 20 years of age is often three to six times higher than that of individuals who are above 20 years of age. There are reports that suggest males are more severely and frequently affected by parasitic diseases (Moirá *et al.*, 2010). Bernin & Loter (2014) assert that schistosome infections are often significantly higher in males than in females. This has been attributed to differences in socio-cultural behaviours among males and females with males engaging more in risk taking behaviours as well as task sharing activities (Moirá *et al.*, 2010). Differences in immune responses between males and females is also likely to play a role in susceptibility differences to schistosome infection (Bahia-Oliveira *et al.*, 1992). A study conducted on mice by Bahia-Oliveira *et al.* (1992) suggests that hormonal differences among males and females play a role towards their differences in susceptibility to schistosome infections. Immunological differences between males and females exist, with females having elevated levels of immune responses when compared to males (Klein & Flanagan, 2016). It has been established that the number of immune cells involved in immunological reactions is higher

in females than in males (Klein & Flanagan, 2016). It has also been established that humoral immune responses are relatively higher in females than in males. Humoral immune responses involve production of antibodies by B-cells (Klein & Flanagan, 2016). It has also been revealed that following exposure to *Schistosoma* parasites, there is often a higher increase of Interleukin 10 (IL-10), Tumour Necrosis Factor alpha (TNF- α) and Interferon gamma (IFN- γ) in females than in males meaning that in such situations, the female immune defence system is at a better defence position than the male (Bernin & Lotter, 2014).



Figure 8: School aged children ‘fishing’ in River Kole (2.302313, 32.68811), a seasonal river in Northern Uganda.

From the image, it is clear to see that school aged children are one of the most at risk demographics because of engaging in behaviour that brings them in contact with waters that are likely infested with schistosome infected snails.

Economic activities in localities that report high prevalence of schistosomiasis constitute rudimentary fishing and farming activities that involve wading into snail infested water bodies (Mulero *et al.*, 2019). Fishing as an occupation and a socio-economic activity is a risk factor to schistosome infections (Bakuza *et al.*, 2017). In epidemiological studies pertaining to schistosomiasis, individuals who engage in fishing as an occupation always exhibit high levels of schistosome infection prevalence compared to the average person in that locality. Sand harvesters are equally at a higher risk of being infected with schistosomes because their occupation involves wading in potentially snail infested waters that are likely to harbour active cercariae (Boelee & Madsen, 2006).

1.1.5 Measurements of schistosome infections

Several parameters are used to measure and establish infection patterns in the context of vector borne diseases and diseases that involve a definitive host and an intermediate host. The most used parameters to measure schistosomiasis are prevalence, incidence, and intensity of infection (Weerakoon *et al.*, 2015).

Prevalence refers to the number of cases of a disease in an entire population (the proportion of individuals in a given population who are infected with a certain disease during a certain period of time). Prevalence can also be defined as the measurement of all the individuals infected by a disease at a particular time (WHO, 2013). Prevalence as a parameter is always expressed in percentage. Given that it is impossible to involve all individuals of a given population in an epidemiological study, prevalence is often regarded as the proportion of infected individuals out of all the subjects involved in the study. Community level prevalence of heavy schistosome infection is currently the basis for morbidity control (WHO, 2013). Mass drug administration (MDA) programmes are currently initiated in communities where it has been ascertained that the prevalence of schistosomiasis in school aged children is higher than 50% (Trienekens *et al.*, 2022).

Incidence on the other hand refers to the number of individuals who have been newly infected with a disease during a particular time (WHO, 2013). Incidence can also be defined as the proportion of individuals who initially were uninfected but became infected over a certain period. Incidence as a measurement of infection is often expressed in percentage as the percentage of new infections per year in relation to the number of at-risk individuals. Incidence as a measurement is very important in monitoring the effectiveness of disease control programmes such as mass drug administration programmes. However, the downside of incidence as a parameter is that it mostly fails to provide accurate results because not all new cases of infections are recognised and reported (WHO, 2013).

Intensity of infection refers to the concentration of pathogens in a host (in the case of schistosomiasis, the number of parasites in a host). The intensity of infection in schistosomiasis is established by counting the number of parasite eggs in urine or faeces from an infected individual. There is always a correlation between worm burden in a definitive host and eggs count in urine and faeces, as far as parasitic infections are concerned. Since establishing the exact number of worms in a definitive host would involve killing the host, counting the number of eggs in urine and faeces has been settled on as the best approach of establishing the intensity of parasitic infections, including schistosomiasis. Intensity of *S. haematobium* infection is classified by the number of schistosome eggs per 10 ml of urine with $n \leq 50$ eggs per 10 ml of urine signifying a light infection and $n \geq 50$ per 10 ml defining a heavy infection (Weerakoon *et al.*, 2015). For the case of *S. mansoni* infection, intensity of infection is normally measured as eggs per gram (EPG) of stool. An infection of 1-99 EPG is defined as a light infection; an infection of 100-399 EPG is regarded as a moderate infection and an infection of $n \geq 400$ EPG is regarded as a heavy infection (WHO, 2002). A study on intensity of schistosome infection has established an association between intensity of schistosome infection and morbidity, the higher the intensity of infection, the higher the morbidity rate (Wiegand *et al.*, 2021). Intensity of infections is always an indicator of morbidity risk. Morbidity is a term that refers to an individual having a disease or showing symptoms of a disease. In the case of schistosome

infections, individuals with high egg counts are likely to be more ill than individuals with low egg counts. Morbidity often plays a very important role as far as disease control programmes are concerned. For instance, after mass drug administration programmes, cases of low egg counts imply the success of the programmes whereas cases of high egg counts imply failure of the programmes (Weerakoon *et al.*, 2015).

1.1.6 Climate change and schistosomiasis

Climate change refers to the change in average weather patterns in a region over a long period and this involves warming temperatures, melting of mountain glaciers, changes in precipitation and rise in sea levels with the greenhouse effect the main driver of climate change. Climate change poses a threat to prevention and control efforts, in the fight against neglected tropical diseases. Climate change that creates conducive conditions that promote the fecundity of causative agents of neglected tropical diseases is likely to jeopardise efforts to eliminate and control neglected tropical diseases (Tidman *et al.*, 2021). It has been established that climate change is likely to instigate emergence of new schistosomiasis transmission foci with outbreaks of schistosomiasis being reported at higher altitudes and latitudes, past previously defined limits. In Uganda, cases of schistosomiasis have been reported recently in glacier lakes, above previously defined limits, suggesting that the higher altitudes have become warmer and conducive for the habitation of *Schistosoma* parasites and snail intermediate hosts (Rubaihayo *et al.*, 2008). New cases of schistosomiasis have also been reported in temperate regions of Europe, suggesting that those regions are becoming warmer and conducive for the transmission of schistosomiasis; this can be attributed to climate change. According to Tidman *et al.* (2021), 2011–2020 was the warmest decade ever with human instigated global warming appearing to increase at the rate of 0.2°C per decade.

Change in land use such as development of new human settlements and clearing fallow land for farming also affects the natural habitat of disease-causing organisms, leading to change in pathogens interactions and subsequent change in disease transmission. Changes in parasite interactions consequentially lead to parasite evolution and parasite evolution directly influences disease control (Mennerat *et al.*, 2010).

In recent years, there has been a growing research interest on the possible effects of climate change on the transmission of infectious diseases and this has involved studies on schistosomiasis (Booth, 2018). The life cycle of *Schistosoma* parasites, constitute stages such as miracidia, primary sporocyst, secondary sporocyst and cercariae that are sensitive to climatic factors such as temperature and water volume (Booth, 2018). Miracidia development is unable to take place at temperatures below 9°C and above 30°C. On the other hand, cercarial shedding is also temperature dependent with minimal shedding taking place outside a 9°C to 26°C temperature range (Kendall & McCullough, 1951). Furthermore, the survival of snail intermediate hosts is dependent on environmental factors, fecundity increasing with temperature until optimum temperature levels (26°C and 31°C). Aestivation, which is a period of dormancy, helps snail intermediate hosts to cope with stressful environmental conditions such as drought and higher temperatures. However, it has been documented that aestivation negatively affects sporocyst development in the snail intermediate host. According to a temperature sensitive model developed by McCreesh & Booth (2014), snail populations are unable to survive outside a temperature range of 15 °C

to 31°C, with a decline in population size outside that temperature range. Therefore, it can be affirmed that suitable temperatures are important for higher levels of schistosome transmission.

In direct relation to humans, the risk of human beings being infected with schistosomes in a lake scenario has been established to be highest at a temperature range of between 15°C and 19°C. On the other hand, in a river setting, human beings are at risk of being infected by schistosomes at a temperature range of between 20.5 °C and 25.0°C with low to no risk of infection at temperatures of 15°C and below and that infection risk increases as temperature increase above the minimum level (McCreesh & Booth, 2014). A deterministic model developed by Mangal *et al.* (2008) suggests that higher temperatures, above 30°C are likely to significantly reduce the intensity and prevalence of schistosome transmission.

Drug resistance continues to be associated with increased temperatures. It has been documented that climate change is likely to alter the population genetics of pathogens and in the process increase cases of drug resistance to chemotherapeutic agents (Burnham, (2021).

Suffice is to state that climate change has a direct impact on the state of any aquatic ecosystem and ideal temperature is vital as far as parasites and snail intermediate hosts' development is concerned. Changes in precipitation patterns, droughts and flooding will most definitely have an impact on the future prevalence of schistosomiasis. However, it is yet to be established whether only climate change has an existential influence on the future transmission of schistosomiasis. McCreesh *et al.* (2015) asserts that other than climate change, non-climatic changes will also play a major role in determining future transmission of schistosome infections. The non-climatic changes include change in land use, increase in human population, change in sanitation infrastructure and change in human-water contact behaviour.

1.2 SCHISTOSOMA MANSONI

Schistosoma mansoni is the most significant *Schistosoma* parasite as far as transmission of human schistosomiasis is concerned (Standley *et al.*, 2012a). *S. mansoni* is solely responsible for 83 million cases out of the current 250 million cases of schistosomiasis (Morgan *et al.*, 2005). Adult *S. mansoni* worms live in the mesenteric veins of the small intestine where they release approximately 20-300 eggs in a day (Jordan *et al.*, 1993). Later, the eggs are passed into the intestines' lumen through blood capillary tubes and subsequently ejected from the body with faeces (WHO, 2013). Often the eggs rupture while in the blood vessels causing lesions. The rupture usually causes bleeding leading to blood in faeces, which is typical symptom of intestinal schistosomiasis (WHO, 2013). Production of eggs is very high in cases where there is no treatment intervention (Jordan *et al.*, 1993). The life expectancy of *S. mansoni* averages twenty years but adult *S. mansoni* can live for up to 40 years in a mammalian host (Harris *et al.*, 1984).

S. mansoni is widely distributed across the globe (Blanchard, 2004). It is the most widely distributed *Schistosoma species*, extensively distributed in South America, the Caribbean, 36 countries in Africa, 7 countries in East Mediterranean and the Middle East (Figure 9). *S. mansoni* is mostly found in places with poor sanitation, because of the parasite's faecal

transmission route (Blanchard, 2004). Adult male *S. mansoni* parasites are white in colour, 0.6 cm to 1.4 cm long and 0.11 cm wide. At the anterior end, male *S. mansoni* have a funnel shaped oral sucker Secor & Colley, (2005). On the other hand, adult female *S. mansoni* are grey in colour, they have a cylindrical body of 1.2cm length and 1.6 cm width Secor & Colley, (2005). *S. mansoni* is mostly found in environments where the inhabiting communities engage in agricultural activities associated with dams and swamps. Irrigation schemes, streams, dams, and lakes are some of the sites that harbour *S. mansoni* parasites (Gryseels *et al.*, 2006).

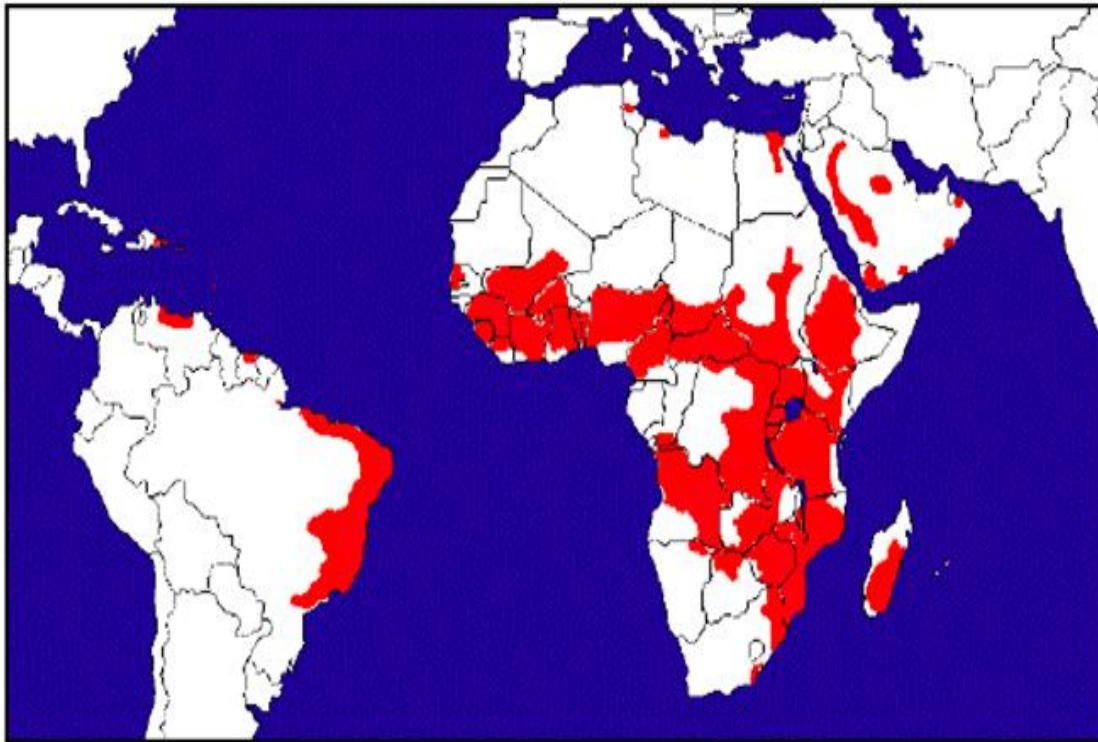


Figure 9: Geographical distribution of *S. mansoni*, courtesy of (Abadi, 2018).

S. mansoni is found in East Africa, West Africa, North Africa, Central Africa, South Africa, South America (Brazil, Suriname and Venezuela), the Caribbean (including Puerto Rico, St Lucia, Guadeloupe, Martinique, Dominican Republic, Antigua and Montserrat) and parts of the Middle East (Abadi, 2018).

Adult worms are tubular in shape and elongate, with a length of 10-20mm (Figure 10). Both male and female adult parasites have ventral and oral suckers. Males are always shorter in size compared to females and they possess a gynaecophoric canal that houses the female adult parasite. The female *S. mansoni* lives in the gynaecophoric canal of the male for the entire of its adult life, except when it moves out to lay eggs in the venules (Chevillon *et al.*, 2012).

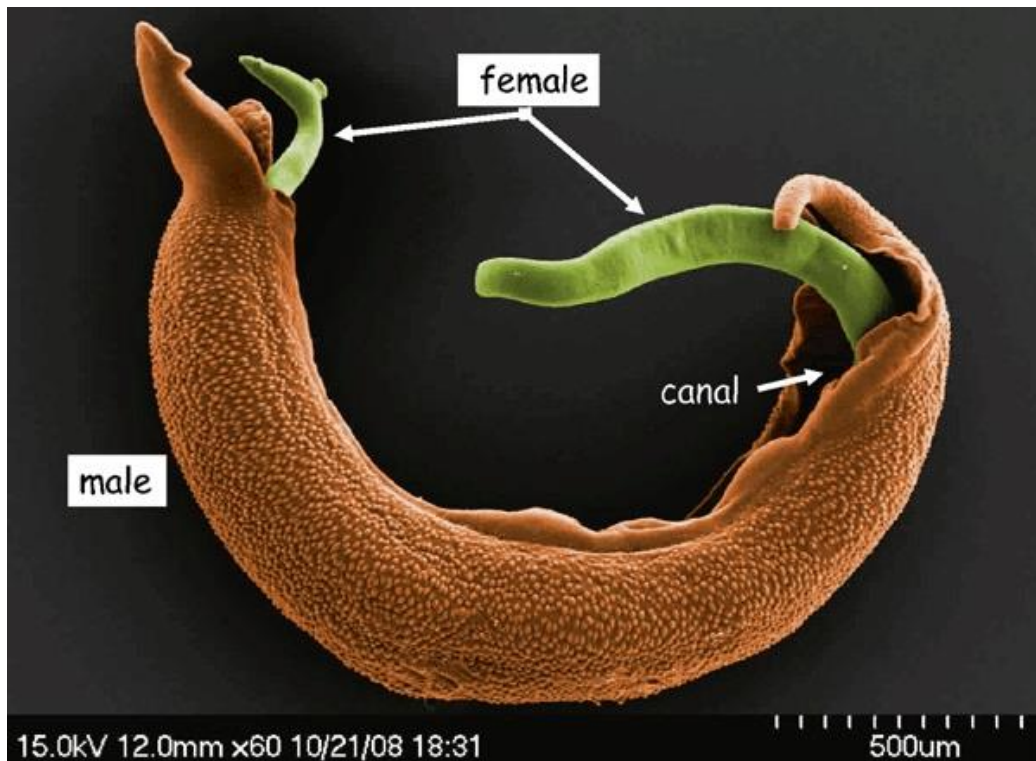


Figure 10: The male is figured in orange in this case whereas the female is figured in green (Chevillon *et al.*, 2012).

1.2.1 Life cycle of *S. mansoni*

The life cycle of *S. mansoni* parasites takes a minimum of 12 weeks and it involves an intermediate host and a definitive host (Gryseels, 2012). *S. mansoni* has two free living stages (cercariae and miracidia) that help the parasite move from an intermediate host to a definitive host and vice-versa (Gryseels, 2012). It takes an average of 35 days between infection of a definitive host by cercaria and the release of eggs by mature *S. mansoni* parasites (Toledo & Fried, 2011). *S. mansoni* eggs are laterally spined; a good distinguishing feature between *S. mansoni* eggs and eggs of other *Schistosoma* species (Figure 11).

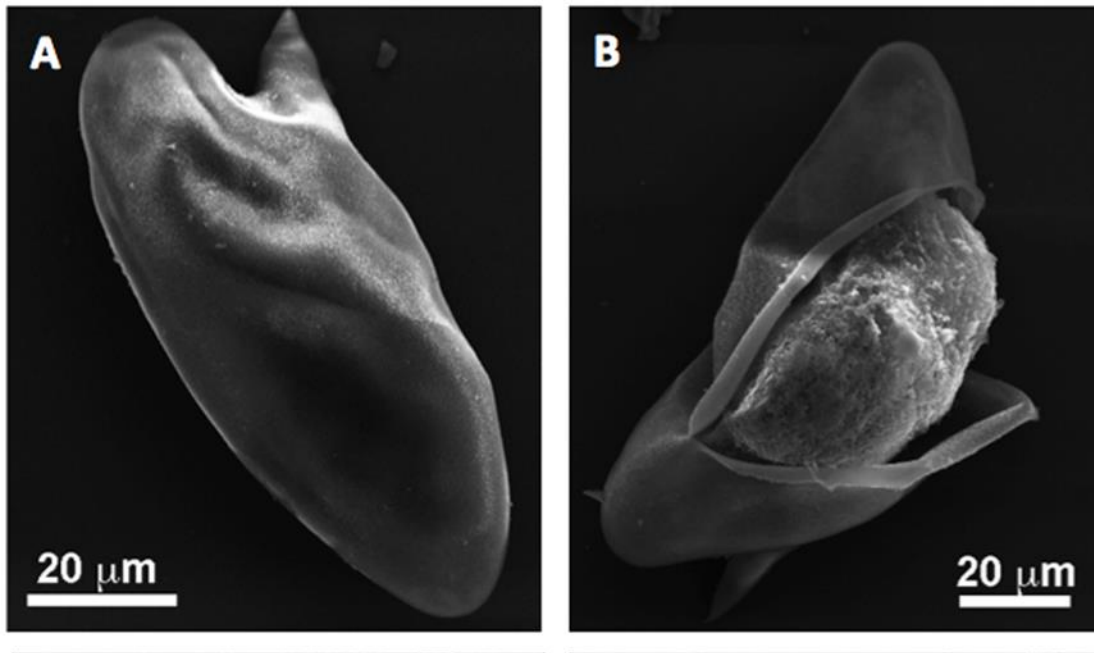


Figure 11: Morphology of *S. mansoni* egg. Panel A shows an intact egg of *S. mansoni* whereas panel B shows *S. mansoni* egg broken with the miracidium still inside the egg (Karl *et al.*, 2013).

When released in freshwater, *S. mansoni* eggs take an average of 5 days to hatch and release miracidia (Toledo & Fried, 2011). At the time of hatching, ciliary action from miracidia within the eggs is promoted. Subsequently, a vent opens on one side of the egg instigating emergence of miracidia, from the egg into the water. Miracidia are free swimming, elliptical larval stages covered with cilia. Miracidium is 70–80 µm in width and 150–180 µm in length (Figure 12). It has four layers of epidermal plates that are covered with numerous cilia with cilia facilitating locomotion. The hatching process occurs in response to light, temperature, change in pH and dilution of faeces with water (Bahia *et al.*, 2006).

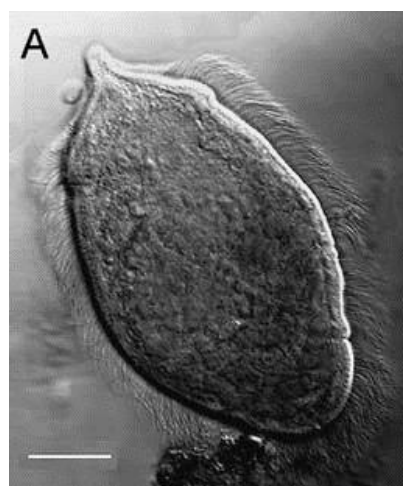


Figure 12: An image of an *S. mansoni* miracidium (courtesy of Bahia *et al.*, 2006).

1.2.1.1 Lifecycle of *S. mansoni* in the intermediate host

To complete the life cycle, miracidia must find a suitable snail host, and this has to happen before it depletes its food storage. Miracidia have an average life span of 16 hours. On locating a potential intermediate host (using phototropic receptors), miracidia swim towards the snail, initially in a straight manner and later in a curved manner (miracidia are highly motile). On coming in contact with the snail, miracidia produce lytic and adhesive enzymes that enable it to attach and penetrate the snail's soft tissues, often the foot. The penetration process takes about 30 minutes to reach completion (Chandler, 1955).

In the snail, the parasite develops into primary sporocysts. Primary sporocysts are formed from germ cells. In the formation of primary sporocysts, ciliated epithelium is shed. Later, sensory, and digestive organs of primary sporocysts are formed. Subsequently, daughter sporocysts are formed through asexual reproduction in the digestive glands of the snail within two weeks (Chandler, 1955). Daughter sporocysts later migrate to the snail's hepatopancreas developing into thousands of new parasites known as cercariae. Thousands of cercariae all of which are capable of infecting the definitive host emerge from one miracidium (a single miracidia can produce thousands of cercariae). This phase of the life cycle of *S. mansoni* lasts up to 7 weeks depending on environmental conditions (Chandler, 1955). It is worthy to mention that *S. mansoni* parasites have a detrimental effect towards the ability of snails to produce eggs and their infection potentially increases the mortality rate among snail populations (Sturrock, 1965).

Cercariae are free swimming larvae, with forked tails that can penetrate the skin of humans (Figure 13). Emergence of cercariae from snails is mediated by ambient temperature and light. Cercariae emergence from the intermediate snail occurs during daytime and it is sunlight and temperature dependent (McCreesh & Booth, 2014). Cercarial shedding of *Schistosoma* parasites that infect human beings is always at its peak between 16°C and 18°C and at noon (a time when sunlight intensity is high in the tropics). On the other hand, cercarial shedding of *Schistosoma* parasites that infect rodents is always at its peak at dusk (Standley *et al.*, 2012b). On average, cercariae production lasts for 4 to 5 hours after which they move towards light, close to the water surface, from where they can infect a definitive host (Wolmarans *et al.*, 2002).

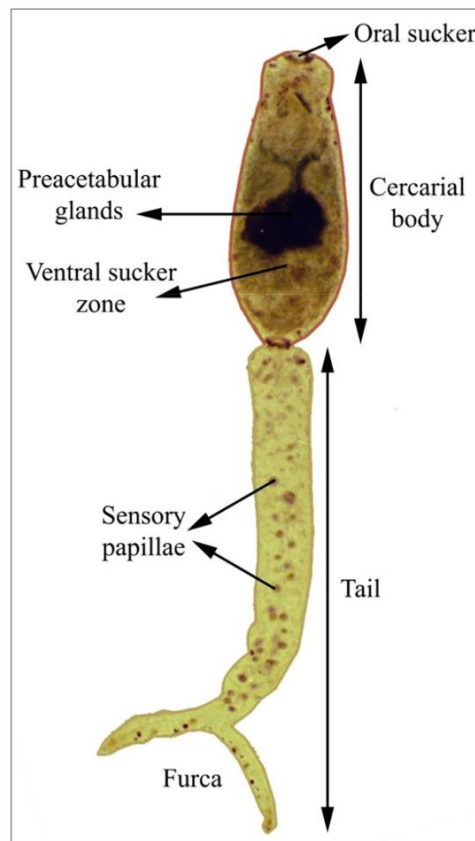


Figure 13: Dorsal view of a *S. mansoni* cercaria observed under the light microscope after a fixation in silver nitrate (~ 330 μm long). It is a swimming larva courtesy of its bifurcated tail. The oral sucker and the ventral sucker (known as acetabulum) facilitate attachment to the tegument of the definitive host. Pre- and post-acetabular glands facilitate host penetration thanks to their proteases. The tail is shed upon penetration (Boissier *et al.*, 2019).

It takes an average of 30 days for cercariae to be released from an infected snail host (Foster, 1964). After their release from the snail intermediate host, cercariae must find a definitive host and penetrate it before they run out of their food reserves and die (Colley *et al.*, 2014). To facilitate the penetration process, cercariae often use proteolytic enzymes to penetrate the skin of definitive hosts. The proteolytic enzymes are produced in the head region of cercariae (Colley *et al.*, 2014).

1.2.1.2 Lifecycle of *S. mansoni* in the definitive host

Cercariae, with the aid of their bifurcated tail propel themselves in water in search of a definitive host with environmental cues helping them locate the definitive host (Nelwan, 2019). On average, it takes around 48 hours outside the snail intermediate host, before cercariae exhaust their food resources (McCreesh & Booth, 2014). Specialized gland cells possessed by cercariae release a mixture of enzymes (proteolytic enzymes, histolytic

enzymes and hyaluronidase enzymes) that digest the stratum corneum (the outermost layer of the skin) enabling penetration into the definitive host (Nelwan, 2019). Cercariae often penetrate the definitive host after a short time of recognizing the human skin (with recognition partly facilitated by arginine secreted by human skin) (McKerrow & Salter, 2002). Penetration involves three stages: attachment to skin, creeping over the skin in search of a suitable penetration site which is mostly a hair follicle and finally penetration into the epidermis with the aid of cytolytic secretions from the cercarial post-acetabular and pre-acetabular glands (Jordan *et al.*, 1993).

In the human definitive host, cercaria undergo development, transforming into endoparasitic larvae (Jordan *et al.*, 1993). The endoparasitic larvae, a migrating schistosomulum remains in the skin for a few days locating a post-capillary venule and later migrates to the lungs where it undergoes further development in preparation for subsequent migration to the liver (Jordan *et al.*, 1993). Schistosomules reach the lungs 5-7 days after penetration of the skin. Approximately 70% of lung stage schistosomules are eliminated by the immune system of human beings once they reach the pulmonary capillaries (Jordan *et al.*, 1993). The remaining 30% reach the liver eight days after penetration of the skin. In the liver, schistosomules develop an oral sucker and use it to feed on red-blood cells.

Skin stage schistosomules develop from cercariae after the latter has lost its tail and penetrated the skin of the definitive host. Lung stage schistosomules develop from skin stage schistosomules after the latter has entered a blood vessel and travelled via the pulmonary artery to the lungs (Figure 14) (McKerrow & Salter, 2002).



A. Skin stage schistosomulum



B. Lung stage schistosomulum

Figure 14: Skin stage schistosomulum and lung stage schistosomulum (Wilson & Coulson, 2009).

Nearly mature adult male *S. mansoni* later pair with longer, cylindrical female *S. mansoni* and the worm pairs then migrate to the mesenteric veins of the small intestines. *S. mansoni* worms reach maturity six to eight weeks after which the female begins to produce eggs with the production of eggs being dependent on the oxidation of fatty acids. In addition, the adult parasites feed on red blood cells of the definitive host (McKerrow & Salter, 2002).

Adult *S. mansoni* pairs located in the mesenteric vessels may produce about 300 eggs in a day with the eggs passing the walls of blood vessels, through the intestinal wall and later passed out in faeces (McKerrow & Salter, 2002). It has been established that the eggs cause damage to the intestinal tract and the liver and subsequent complications arise because of inflammatory reactions to the eggs (Gryseels, 2006). Adult *S. mansoni* parasites live an average of 3-11 years inside the definitive host but there are cases where the parasites live for as long as 40 years in the definitive host (Colley *et al.*, 2014). Figure 15 shows the life cycle of *S. mansoni* parasites.

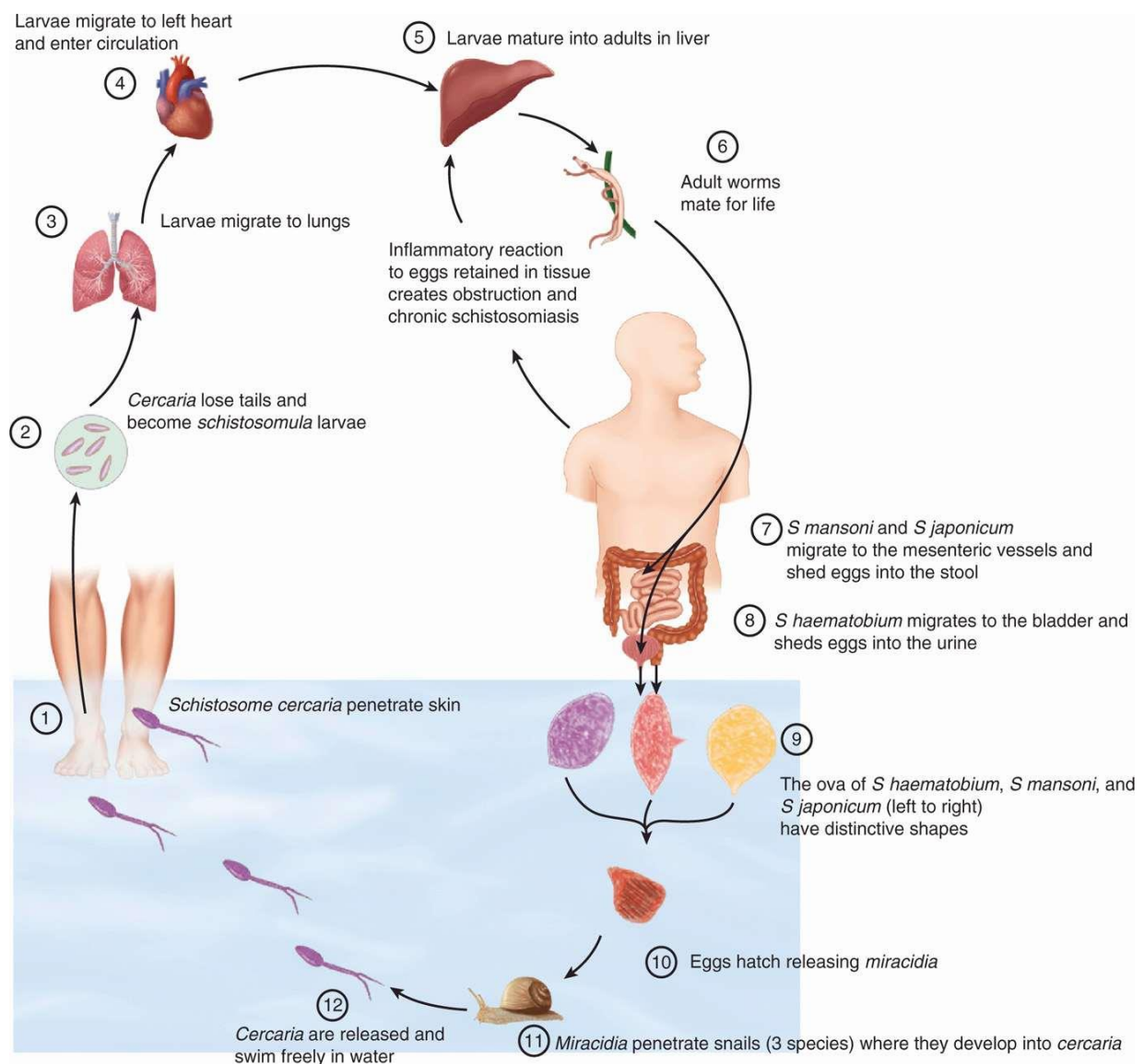


Figure 15: The life cycle of *S. mansoni* (WHO, 2013).

1.3 SCHISTOSOMIASIS PREVENTION AND CONTROL

Prevention and control strategies in the fight against schistosomiasis include preventive chemotherapy, snail control, health education and improved hygiene and sanitation (Sokolow *et al.*, 2016). There are countries in the world that have been successful in preventing and controlling the transmission of schistosomiasis and this include several countries in Latin America, Caribbean, South America, and Asia. The countries that have successfully controlled the transmission of human intestinal schistosomiasis are Japan, Martinique, Montserrat, China, Philippines, Egypt, Brazil, Iran, Lebanon, Malaysia, Thailand, Tunisia, and Turkey (Tchuem *et al.*, 2017). Out of the 'success stories', Egypt, the Philippines, China and Brazil are regarded as 'model' countries because of their great achievements in reducing the prevalence of schistosomiasis. On the other hand, Morocco, Jordan and Antigua are regarded as potential 'success' stories as far as schistosomiasis prevention and control are concerned because of the advancements they have made in controlling the spread of the disease (Sokolow *et al.*, 2016). Since 1991, no new cases of schistosomiasis have been reported in Iran, Morocco, Tunisia, Turkey, Japan, and Malaysia (Hewitt & Willingham, 2019).

There are also countries where it has been claimed that schistosomiasis has been eliminated (a total of 19 countries) but evaluation needs to be done to verify if interruption of transmission has truly been achieved as claimed; these countries are Algeria, Antigua, Djibouti, Dominican Republic, Guadeloupe, Iran, India, Japan, Jordan, Lebanon, Malaysia, Martinique, Montserrat, Morocco, Thailand, Tunisia, Turkey, and Puerto Rico (WHO, 2013). There are also countries that have made great strides in schistosomiasis control (Iraq, Libya, Oman, Saudi Arabia, Saint Lucia, Suriname, and Syria) but there is a need to update information about the status of schistosomiasis in those countries for planning and implementation purposes (WHO, 2013).

1.3.1 Preventive chemotherapy

Preventive chemotherapy refers to the regular, deliberate, and large-scale administration of drugs to high-risk individuals of a population or the entire population of populations living in a disease endemic area (Montresor *et al.*, 2012). Preventive chemotherapy is the most popular intervention approach used to prevent and control the spread of schistosomiasis. Preventive chemotherapy is normally executed after several surveys have been conducted in an area and it has been proven beyond reasonable doubt that the burden posed by the disease to individuals living in that area is relatively high (Montresor *et al.*, 2012). Preventive chemotherapy approach that involves an entire population of a given area is called mass drug administration. On the other hand, preventive chemotherapy approach that involves a specific demographic of a population such as fishermen and school aged children is called targeted treatment. Then again, there is selective treatment which refers to preventive chemotherapy that involves individuals who keep on testing positive when screened. Given that schistosomiasis, together with other neglected tropical diseases are diseases of poverty and afflict individuals who live in similar geographical locations, mass drug administration programmes for the diseases is sometimes done collectively. This implies that preventive chemotherapy of schistosomiasis is sometimes done alongside preventive chemotherapy of diseases such as soil transmitted helminthiasis, lymphatic filariasis, onchocerciasis and trachoma. Ivermectin is the drug of choice for lymphatic

filariasis and onchocerciasis. Albendazole is the drug of choice for soil transmitted helminthiasis and azithromycin the drug of choice for trachoma (Montresor *et al.*, 2012).

According to the World Health Organisation (2013), 52 countries require preventive chemotherapy intervention in the fight against schistosomiasis and these are: Angola, Benin, Botswana, Burkina Faso, Burundi, Brazil, Cambodia, Central African Republic, Chad, China, Congo, Cote Ivory Coast, Democratic Republic of Congo, Egypt, Eritrea, Ethiopia, Gabon, Gambia, Ghana, Guinea, Guinea- Bissau, Laos, Kenya, Liberia, Madagascar, Malawi, Mali, Mauritania, Mozambique, Namibia, Niger, Nigeria, Philippines, Rwanda, , Sao Tome and Principe, Senegal, Sierra Leone, Somalia, South Africa, South Sudan, Swaziland, Togo, Uganda, United Republic of Tanzania, Venezuela, Yemen and Zambia.

Praziquantel is the drug recommended by the World Health Organization for the treatment of schistosomiasis. It was developed by Bayer (a German pharmaceutical company) in the 1970's. Before praziquantel was adopted as the drug of choice, other drugs had been tried out but due to them being less effective and having potentially deleterious side effects, they were relegated to the periphery. Tartar emetic is one of the first drug that was used in the treatment of schistosomiasis. As early as 1913, tartar emetic was being used to treat schistosomiasis. Other drugs that have been used in schistosomiasis control include oxamniquine and artemisinin (Montresor *et al.*, 2012).

Praziquantel functions by killing adult *Schistosoma* parasites and at the same time stopping them from laying eggs. Praziquantel can be prescribed to any age group, has low toxicity and a good tolerance and it is administered orally by a single dose. Praziquantel instigates entrance of calcium ions into muscles of mature forms of the *Schistosoma* parasites prompting non - physiological contraction which results to paralysis and death (Gryseels *et al.*, 1994). It has been established that praziquantel is safe for pregnant mothers during their first trimester (Colley *et al.*, 2014). However, one drawback of praziquantel is that the drug is inactive against immature worms, meaning that the juvenile worms could mature and commence production of eggs after chemotherapy has been completed (Bergquist & Elmorshedy, 2018). A new recommended approach to address the limitation of praziquantel, of killing only adult worms is that of using a synergistic drug that combines artemether with praziquantel. Artemether kills immature worms whereas praziquantel kills adult worms. Using the synergistic drug would ensure that both immature and adult worms are killed (Bergquist & Elmorshedy, 2018).

Oxamniquine is regarded as the second drug of choice in schistosomiasis control but there are several factors that make oxamniquine less attractive, compared to praziquantel. The first reason is that oxamniquine is expensive and second; it is only effective against one *Schistosoma* parasite, *S. mansoni*. The third factor that makes oxamniquine less attractive is that it does not eliminate male *Schistosoma* parasites from the body. The drug works by orchestrating shifting of worms from the mesenteric veins to the liver where the males are retained. In addition, oxamniquine inhibits female worms from releasing eggs and as a result prevent hepatic granuloma formation (Gryseels *et al.*, 1994).

Artemisinin is the newest drug to be developed and used for the treatment of schistosomiasis (Hommel, 2008). The drug has been used for a long time in the treatment

of malaria. It is active against immature stages of *Schistosoma* parasite. However, it has not been widely adopted for the treatment and control of schistosomiasis. Artemisinin is naturally obtained from leaves of *Artemisia annua* plant, a sesquiterpene lactone with a peroxide group. Anti-schistosomal properties of Artemisinin were discovered by Chinese scientists in the 1980's. Artemisinin derivatives include artemether and artesunate with dihydroartemisinin being the primary active metabolite (Hommel, 2008).

Preventive chemotherapy benefits are reduction of intensity of schistosome infections, morbidity and mortality associated with schistosomiasis. In addition, physical fitness, linear growth, weight status, nutritional status and cognitive functions are improved courtesy of preventive chemotherapy. Furthermore, preventive chemotherapy increases people's appetite, subsequently improving their physical health, work productivity and lifespan. Moreover, preventive chemotherapy reduces the prevalence of bloody diarrhoea in human intestinal schistosomiasis (Bergquist & Elmorshedy, 2018).

In conclusion, preventive chemotherapy has played a major role in preventing and controlling transmission of schistosomiasis. Preventive chemotherapy has made tremendous progress in reducing morbidity and mortality associated with schistosome infection. However, reinfection of schistosome parasites in treated individuals continues to undermine preventive chemotherapy efforts. In many endemic areas where infection levels drop after mass drug administration programmes, infection levels often go up after months or years of no treatment (Colley *et al.*, 2014). Moreover, there has been concern over development of *Schistosoma* parasites' drug resistance to praziquantel. The resistance has been explained by observation of low cure rates with time (Gryseels *et al.*, 1994). The success of preventive chemotherapy also continues being jeopardised because of the refusal of some individuals in endemic communities to take medication and also, the mobile nature of human beings has made it impossible for preventive chemotherapy programmes to reach all intended people. There are many cases where public health officials administering praziquantel have failed to meet all the desired individuals in a particular community. For instance, fishermen in most African villages spend a lot of time from their homes and as a result miss out on many preventive chemotherapy programmes (Parker & Allen, 2011).

1.3.2 Mass Drug Administration programmes

Mass drug administration programmes are part of preventive chemotherapy efforts. Mass drug administration using Praziquantel is the first priority recommended by the World Health Organisation as far preventing and controlling transmission of schistosomiasis is concerned (Colley *et al.*, 2014). The decision to use mass drug administration as the recommended intervention approach was arrived at in 1985 by a WHO expert committee. The dosage of Praziquantel that is recommended by WHO is 40 mg/kg (Kibira *et al.*, 2019).

Mass drug administration programmes mostly target school aged children (Bergquist & Elmorshedy, 2018). The programmes always focus on school-aged children as they are thought to be the most infected because of their proclivity to swim and play in waters infested with schistosome infected snails and because they are an easy to target demographic because they can easily be found together in one place, at school. The World Health Organisation recommends that in an any given location, infection levels in school

aged children should be used to determine the prevalence of the disease in that particular area (Colley *et al.*, 2014).

A community is regarded as a high-risk community for schistosomiasis prevalence if it has been established by parasitological diagnostic methods that more than 50% of school-aged children are infected (Toor *et al.*, 2018). A community is regarded as a moderate risk community for schistosomiasis prevalence if it has been established by parasitological methods that between 10 and 50% of school aged children are infected. A community is regarded as low risk community for schistosomiasis prevalence if it has been by parasitological methods that less than 10% of school aged children are infected (Toor *et al.*, 2018). In low-risk areas, WHO recommends that that school aged children (aged between 5 and 14 years) and adults suspected to be infected should be treated once in every three years. In moderate risk areas, WHO recommends that school aged children and adults who are at risk of being infected should be treated once in every 2 years. In high-risk areas, WHO recommends that school aged children and adults who are infected should be treated annually (Toor *et al.*, 2018). Notwithstanding, in recent years there has been a heavy campaign to include pre-school aged children and adults in MDAs (Kura *et al.*, 2021).

The World Health Organization intends to eliminate schistosomiasis as a public health problem by 2025, and this involve reducing cases of heavy worm burden schistosomiasis among school aged children to less than 1% (Toor *et al.*, 2018). It has been proven that mass drug administration programmes that involve both school aged children and adults are very effective in bringing out significant reductions as far controlling morbidity, intensity and prevalence of schistosomiasis is concerned (Toor *et al.*, 2018).

Mass drug administration programmes in Africa have not been successful because of several reasons (Adriko *et al.*, 2018). First, there is limited supply of praziquantel drugs because not all times are the drugs freely supplied and additionally, there is also scarcity of funds in Africa to purchase the drug. This means that there are more individuals that are supposed to be treated than the drugs available for treatment purposes (Adriko *et al.*, 2018). Secondly, most of the programmes have only been targeting school-aged children. Mass drug administration programmes should not only target school aged children, they should also target adults because *Schistosoma* parasites burden would remain in any community if pre-school aged children and young adults are not treated (Toor *et al.*, 2018). Adriko *et al.* (2018) reveals that many people in areas that have been under mass drug administration programmes do not take part in the programmes as widely expected and believed. In a study conducted in Mayuge district Uganda, an endemic district, 49.5% of the people that were interviewed reported that they have never taken praziquantel in their lives even though the area has undergone several mass drug administration programmes. Furthermore, 49.2% of the individuals stated that they had never been offered Praziquantel. Out of all the individuals who had never taken the drug, 21.4% of them stated that they have never taken the drug because they were away when the mass drug administration programmes were operational. Thirdly, preventive chemotherapy does not stop re-infection and there is growing concern of drug resistance to praziquantel (Crellen *et al.*, 2016; Faust *et al.*, 2019).

A study conducted by Coulibaly *et al.* (2018) with the aim of understanding mass drug administration programmes in Ivory Coast reveals that the programmes are associated with low treatment coverage rates. Work related activities was the main reason for low treatment coverage rates. Individuals were not willing to sacrifice their work-related activities, such as taking part in farming activities, to take part in mass drug administration programmes. Praziquantel's bitter taste was the other reason some individuals shied away from taking the drug. Strangely, more than 75% of individuals who participated in previous drug administration programmes affirmed that they would not take part in future drug administration programmes because of praziquantel's bitter taste and its perceived adverse effects.

Proper communication is likely to play a major role in improving the success of mass drug administration programmes (Bergquist & Elmorshedy, 2018). Communities should be informed in advance about scheduled mass drug administration programmes by using local authorities and leaders to relay such information. Proper communication would allay misunderstandings, suspicions, fears and rumours about mass drug administration programmes. There is also the need for adept communication strategies in readiness for preventive chemotherapy programmes. Communities should be informed about the possible side effects that may arise because of taking the drug (Bergquist & Elmorshedy, 2018).

It is also worthy to mention that for mass drug administration to be successful, it has to be done persistently, without ceasing. Effects of mass drug administration on transmission of schistosomiasis are temporary and studies have proved that ceasing drug administration can return prevalence and intensity of infection to baseline levels. Moreover, schistosome eggs and larval stages of the parasite can survive for a long time in the environment thus creating an opportunity for re-infection after mass drug administration (Campbell *et al.*, 2014). Furthermore, there are sections of the population that do not take part in mass administration programmes and as a result, serve as reservoir for reinfection. It therefore means that the treatment should not only be regular but should also reach all members of a given endemic population for it to yield desirable results. Though desirable, the reality is that it is almost impossible to convince everyone in an endemic population to take part in preventive chemotherapy programmes (Campbell *et al.*, 2014).

1.3.3 Snail control

Snail control in the fight against schistosomiasis involves use of natural predators that feed on snail intermediate hosts, use of competitor snails that are highly invasive (compete with the intermediate hosts for food and displace them from their habitats) and use of molluscicides (chemicals that demonstrate antagonistic activities against the snails (Wu *et al.*, 2019). Eliminating snail intermediate hosts means that *Schistosoma* parasites will be unable to complete their life cycle thus transmission of schistosomiasis will be curtailed. Snails can also be eliminated from a given location by manually removing them by hand. Clearance of vegetation in habitats populated by the intermediate hosts can also be done to control snail populations. Snails are highly dependent on vegetation in their habitats for food and anchorage, therefore, clearance of vegetation thwarts their survival (Wu *et al.*, 2019).

Molluscivorous fish are sometimes also used to control populations of the intermediate hosts. *Astatoreochromis alluaudi* is an example of a fish that has been well documented in field trials in Kenya and Cameroon with an aim of controlling the population of snail intermediate hosts. However, it was realised that the fish has a low reproduction rate and not appropriate in controlling snail populations in the long term (Slootweg *et al.*, 1994). Crayfish have also been used to control populations of snail intermediate hosts by being fed on the snails thus reducing the population of the intermediate hosts (Loker *et al.*, 1991).

Use of competitor snails is another way of controlling the population of snail intermediate hosts. Natural enemies of *Biomphalaria* snails such as *Marisa* snail that competes with *Biomphalaria* snails for food and eats their eggs can be used to control *Biomphalaria* snail populations. *Marisa* species is widely distributed in South America, Africa, Central America and the Caribbean. Introduction of *Marisa* snails in new habitats occupied by *Biomphalaria* snails has played a major role in decimating *Biomphalaria* snail populations (Cedeno-Leon & Thomas, 1982). *Melanooides tuberculata*, a highly invasive snail can also be used to control the population of *Biomphalaria* snails because it has been proven to be very effective in displacing *Biomphalaria* snails from their habitats (Gashaw *et al.*, 2008; Pointier, 1993).

Use of molluscicides can be used in snail control. Molluscicides are chemicals that have the ability to kill snails. Molluscicides are used in agriculture to control gastropod pests which include slugs and snails. Molluscicides act on different enzymes, chiefly those involved in carbohydrate metabolism and respiration. This in turn affects energy production since gastropods primarily utilize carbohydrates for energy production. Failure of the snails to properly engage in respiration and metabolism finally leads to the death of the snails (Kajihara *et al.*, 1979). Molluscicides that have in the past been used to control snail populations include Copper Sulphate, Nicotinic anilide, Niclosamide, Sodium 2, 5 dichloro-4-bromophenol and chemicals that are generally classified as acetylcholinesterase inhibitors (Kajihara *et al.*, 1979). Sodium 2, 5 dichloro-4-bromophenol and copper sulphate were popular in the 1940's and 1950's for snail control. In later years, from 1961, Niclosamide became the most frequently used molluscicide with the perception that it is a compound with low toxicity to human beings and livestock and one with the ability to kill snails and their eggs at low concentrations (King *et al.*, 2015). Currently, Niclosamide is the molluscicide recommended by the World Health Organisation for snail control. It is the only molluscicide whose effectiveness has been completely evaluated and approved. However, Niclosamide, is expensive, has poor water solubility and is harmful to non-target organisms such as fish (Aladesanmi, 2007).

In recent years, plants that possess molluscicidal properties have gained popularity as potential replacements to Niclosamide because it is widely believed that they are environmentally friendly, safe to non-target organisms, safe to use and cost effective. In addition, it has been proven that in addition to some of those plants having molluscicidal properties, they possess antischistosomal properties against larval stages of schistosomes (Magero *et al.*, 2015). Phytochemicals present in the plants are potentially responsible for molluscicidal and antischistosomal properties of the plants. Some of the ethnomedicinal plants that have successfully been used for molluscicidal and antischistosomal activities include *Bridelia micrantha*, *Ocimum americanum*, *Solanum mammosum* and *Eurphobia milli* (Magero *et al.* 2015; Schall, 2010; Mkoji *et al.* 1989).

1.3.4 Environmental management

Environmental management in schistosomiasis control refers to efforts used to modify the environment in such a manner that makes it not conducive for the habitation and survival of schistosome parasites as well as the snail intermediate hosts (Abe *et al.*, 2018). Courtesy of poor environmental management, the environment in most countries where schistosomiasis is endemic is in a manner that encourages the habitation of the parasites and the intermediate hosts.

Environmental management practices centred around engineering, such as cementing water canals, draining wetlands and building bridges have played a major role in controlling the transmission of schistosomiasis (Sokolow *et al.*, 2016). However, according to Sokolow *et al.* (2016), engineering controls cannot significantly control transmission of schistosomiasis; the engineering controls are only beneficial when they become integrated with other control interventions such as snail control and preventive chemotherapy.

Clearing of vegetation in aquatic habitats is one measure that can help in eliminating snail intermediate hosts in such environments. Aquatic plants in water bodies where schistosomiasis is endemic are important, they are egg laying sites, they are a source of food, and they also provide shelter to the snails. Clearing the vegetation in such habitats makes the habitat unfriendly to the survival of the snails, possibly leading to their extermination (Abe *et al.*, 2018).

1.3.5 Public health education

Public health education is a strategy that can be used to prevent and control the transmission of schistosomiasis. Health education aims to ensure that communities living in schistosome endemic areas are educated about the life cycle of schistosomes and the transmission dynamics of the disease so that they make wise choices that would ensure they cease from behaviours that are likely to make them contract the disease (Tchuem *et al.*, 2017).

Knowledge about the life cycle of *Schistosoma* parasites should be imparted in people living in endemic areas to prevent infections caused by ignorance (Bundy & Guyatt, 1996). Communities should also be taught on importance of using clean water supplies and pit latrines. People in endemic areas should be taught on importance of using footwear while working in irrigation schemes. It has been documented that many individuals who live around water bodies where schistosomiasis is endemic have little knowledge about the life cycle of schistosomes and the importance of snail intermediate hosts in the transmission of the parasites (Abe *et al.*, 2018). These very people also have little knowledge on the danger that snail intermediate hosts pose to their health. People who live in endemic areas can be very helpful in schistosomiasis control when they are knowledgeable enough about schistosomes and the snail intermediate hosts. They can be entrusted with the responsibility of reporting the presence of snails to relevant public health officials and in doing so, proactive efforts can be taken to curtail transmission of schistosomiasis (Abe *et al.*, 2018).

Ignorant beliefs about schistosomiasis can and should be demystified by public health education. There are beliefs some rural folk in Africa hold about schistosomiasis that

jeopardises schistosomiasis prevention and control efforts. There are rural folk who believe that they can contract the disease by sharing a bathroom with an individual who is infected with schistosomiasis. Furthermore, there are individuals who believe that a person can contract schistosomiasis through witchcraft (Kibira *et al.*, 2019).

1.3.6 Improved hygiene and sanitation

The tenet of improved hygiene and sanitation is to improve communities access to clean and safe water supplies. If people in low resource settings are provided with clean water supplies, they will not be compelled to use unsafe water for bathing, washing, and cooking (Colley *et al.*, 2014). Piped water supplies should be provided by the government or the relevant authorities to discourage people from going to streams and rivers in search of water which may be infested with schistosome infected snails (Kloos *et al.*, 2008).

Provision of portable water would ensure that at risk water contact is reduced and thus reduced schistosomiasis transmission (Colley *et al.*, 2014). A study by Grimes *et al.* (2014) confirmed that safe water supplies are directly linked to few and reduced cases of schistosomiasis. The very study also confirmed that adequate sanitation is directly linked to few cases of schistosomiasis.

There needs to be construction of toilets in communities where schistosomiasis is endemic, to discourage people from those communities from defecating and urinating in the open. Lack of sanitary facilities in areas where schistosomiasis is endemic plays a major role in increasing easy access of schistosomes to humans and snails through urine and faeces from already infected individuals (Abe *et al.*, 2018).

In conclusion, intervention approaches to prevent and control transmission of schistosomiasis in Africa have made great strides to reduce morbidity and mortality associated with the disease on the continent but more needs to be done to achieve better results. There is need for stronger political commitment in the fight against schistosomiasis. Stronger political commitment would ensure that adequate financial resources are allocated to support control and elimination programmes, and this would also ensure the issue of scarcity of Praziquantel drugs is an issue of the past. There is also a need for in country coordination as well as international coordination among stakeholders involved in schistosomiasis prevention and control for purposes of ensuring integrated approaches are embraced. There are several public health experts who posit that schistosomiasis control and prevention should not involve only one intervention approach but an integrated intervention approach that involves preventive chemotherapy, snail control, improved sanitation, improved water supplies, mass education and behavioural changes of individuals who live in endemic areas.

1.4 DETECTION AND DIAGNOSIS OF SCHISTOSOME INFECTIONS

Detection and diagnosis of schistosome infection is important in coming up with appropriate prevention and control strategies. Given that schistosomiasis is more prevalent in low resource settings, it is important to develop diagnostic tools that are applicable to rural settings. Diagnostic tests ought to exhibit high sensitivity and specificity so that individuals who are infected are not missed out in both screening and diagnostic processes (Weerakoon *et al.*, 2015). An ideal diagnostic test should be one that is inexpensive in terms of processing of samples, cost of equipment, reagents, and field

applicability (Weerakoon *et al.*, 2015). Effective diagnosis is vital in development of appropriate control programmes. Effective diagnosis is important in case detection, in areas where the prevalence of schistosomiasis is high as well as in case detection in areas where schistosomiasis prevalence is low (Weerakoon *et al.*, 2015).

Diagnostic tests that are used to diagnose schistosomiasis can generally be categorised into three main groups: parasitological diagnostic tests, immunological diagnostic tests and molecular detection tests. Immunological and molecular diagnostic tests that are lab based are generally more expensive due to the relatively high cost of purchasing equipment, tools, instruments, and reagents (Weerakoon *et al.*, 2015).

1.4.1 Parasitological diagnostic tests

Parasitological methods work under the principle of detecting schistosome eggs in stool and urine of patients or persons suspected to be infected with schistosomes.

Parasitological methods include Kato-Katz technique, urine filtration technique and FLOTAC (Bergquist *et al.*, 2009). Kato Katz technique, a microscopy-based method that was developed in 1972 is the most widely used parasitological detection method as far as schistosomiasis diagnosis is concerned (Katz *et al.*, 1972). Other parasitological diagnostic methods that are used sometimes to diagnose schistosomiasis infections include centrifugation, salt flotation, formol-ether sedimentation and FLOTAC (Weerakoon *et al.*, 2015). A urine filtration technique that uses nylon, polycarbonate filters or paper is the standard method for detection of urogenital schistosomiasis. FLOTAC, a new technique for stool examination has gained popularity over the years and it is arguably a better alternative to Kato- Katz technique because of its relatively heightened sensitivity. (Bergquist *et al.*, 2009).

1.4.1.1 Kato Katz technique

Kato Katz technique has for a long time been the gold standard for the detection of human intestinal schistosomiasis (Gryseels, 2012). Dr Katsuya Kato, a Japanese scientist, developed Kato- Katz technique in 1954. Kato-Katz technique is the most used parasitological method in the diagnosis of schistosomiasis, and it is the diagnostic test recommended by the World Health Organisation in the diagnosis of intestinal schistosomiasis (Weerakoon *et al.*, 2015). The process involves taking a stool sample, preparing a thick smear, and observing it under a microscope. The standardised volume of stool sample that is used in preparation of a Kato Katz smear is 40 milligrams. The cover slide that is used in the Kato Katz technique is often glycerine infused. The smear is then observed under a microscope, looking for *S. mansoni* eggs, characterised by a lateral spine at the posterior end (Weerakoon *et al.*, 2015). The Kato Katz technique is relatively cheap compared to other diagnostic methods. It is simple and can readily be used in field conditions (Weerakoon *et al.*, 2015).

Nevertheless, the sensitivity of this test is low in cases where the intensity of infections is low and the prevalence low (Weerakoon *et al.*, 2015). As a result, the intensity of infection can easily be underreported and thus provide a gross underestimation of disease prevalence. Technical support is mandatory in conducting Kato Katz and this is likely to increase the total expenditure of conducting the Kato Katz technique, more so in cases where the technical staff has to be remunerated (Weerakoon *et al.*, 2015).

1.4.1.2 Urine filtration technique

Urine filtration technique is an inexpensive diagnostic test that filters urine suspected to contain eggs of *S. haematobium* followed by visualization of those eggs under light microscopy. This technique is the gold standard as far as the diagnosis of *S. haematobium* is concerned (Ephraim *et al.*, 2014).

1.4.1.3 FLOTAC technique

FLOTAC technique is a diagnostic method that takes advantages of floatation and sedimentation properties of parasitic elements (Cringoli, 2006). This technique was developed by Cringoli, an Italian parasitologist and it works on the premise of centrifugation of stool samples. This technique orchestrates floatation of parasitic elements onto the surface of a stool/ faecal matter suspension; with parasitic elements here being parasitic eggs and larvae. Once on the surface, the parasitic elements can be transferred to a microscopic slide from where the elements can be observed directly by microscopy (Cringoli, 2006).

The FLOTAC method is appropriate in areas where poly- parasitism exists (Cringoli, 2006). The method can be used to detect multiple parasitic species from a single stool sample. For instance, in cases where soil transmitted helminthiasis coexist with schistosome infections, the FLOTAC technique would be an ideal diagnostic method. The FLOTAC technique has been demonstrated to exhibit a higher level of sensitivity when compared to the Kato Katz technique (Knopp *et al.*, 2011).

1.4.1.4 Cercarial shedding (to detect schistosome infection in intermediate hosts)

Cercarial shedding in snails is the most conventional method that has for many years been used to detect schistosome infections in snail intermediate hosts (Weerakoon *et al.*, 2015). Cercarial shedding involves exposing snails suspected to be infected with schistosomes to sunlight under field conditions, allowing emergence of cercaria from the snails (cercariae are phototrophic, they are attracted to light and exposure of the snails to sunlight under the right environmental temperatures, leads to emergence of cercariae from the snails). Normally, the snails are washed and put in wells containing habitat water; when cercariae emerge, they can be viewed moving in the water under microscopy (Wolmarans *et al.*, 2002). In a laboratory setting, cercarial shedding involves exposing snails to artificial light of about 60 Watts (that mimics natural sunlight) to instigate the snails to shed cercariae and subsequently observations made by microscopy (6-12 hours after exposure to light) to identify the cercariae. The limitation of cercarial shedding is that it can only identify patent infection, meaning that snails with prepatent infection are treated as uninfected snails (Bakuza *et al.*, 2017) and it is also time and energy consuming in the sense that one has to wait for up to 12 hours to ascertain full emergence of cercariae (Nwoko *et al.*, 2021).

In cases where the parasite burden is low, cercarial shedding may fail to detect schistosome infections because in such cases cercaria may not be shed as expected. Cercarial shedding often occurs 25- 30 days after infection and therefore, in cases where the parasite is still under development in the snail as primary or daughter sporocysts, cercarial shedding method may yield false negative results as far as schistosome infection in the intermediate host is concerned. Cercarial shedding is also unable to yield species specific results for parasites that would have infected the snails (Weerakoon *et al.*, 2015).

1.4.2 Immunological diagnostic tests

Immunological methods work by detecting antibodies and/or antigens in urine, blood, serum, or sputum samples (Weerakoon *et al.*, 2015). Immunological diagnostic tests often come in handy in cases where parasitological tests produce false negative results for schistosome infection that are light in nature (Colley *et al.*, 2014). Parasitological diagnostic tests are likely to give false negative results in cases where schistosome infections are of light intensity, more so in areas that have lately been covered by mass drug administration programmes (Colley *et al.*, 2014). Rapid immunodiagnostic tests that are applicable in the field are appropriate, but their limitation is that using them can be expensive as far as processing samples is concerned (Weerakoon *et al.*, 2015).

1.4.2.1 Antibody detection diagnostic tests

Antibody detection as an immunological test has been used to detect schistosomiasis in cases where patients have low egg numbers in their body systems, where the intensity of infection is low. Antibody detection diagnosis tests utilize *Schistosoma* antigens. In antibody detection tests, a patient's serum is often exposed to *Schistosoma* antigens and production of a precipitate is interpreted as a positive sign for the presence of *Schistosoma* eggs in the patient's body system (Weerakoon *et al.*, 2015). One major drawback of antibody detection tests is that serology is unable to properly differentiate active infections and past exposures (Colley *et al.*, 2014).

ELISA (Enzyme Linked Immunosorbent Assay) can be used to test for the presence of *Schistosoma* antibodies in an individual by crossing the individual's serum with antigens that have been extracted from different life cycles of *Schistosoma* parasites. A reaction between the different antigens from the different life cycles of *Schistosoma* parasites would be an indicator of specificity in terms of the life stage or the type of *Schistosoma* parasite present in a particular patient or situation. In recent years, purified antigens have been developed to address the issue of cross reactivity that used to exist with the use of whole parasite extracts (Weerakoon *et al.*, 2015).

1.4.2.2 Antigen detection diagnostic tests

Detection of *Schistosoma* antigens in the blood of patients has over the years gained prominence as an appropriate diagnostic approach (Weerakoon *et al.*, 2015). Circulating Anodic Antigens (CAA) and Circulating Cathodic Antigens (CCA) have been the targets of antigen detection tests. Both CCA and CAA can be detected in a patient's blood, 3 weeks after infection. Furthermore, CCA and CAA can also be detected in a person's urine (Weerakoon *et al.*, 2015).

A lateral flow cassette assay has been developed to diagnose intestinal schistosomiasis, more so the type caused by *S. mansoni* (Figure 16) (Weerakoon *et al.*, 2015). The lateral flow cassette assay exhibits high sensitivity when compared to the Kato-Katz assay (Colley *et al.*, 2014). On the other hand, the CCA test has exhibited several shortcomings in detection of urinary schistosomiasis (Weerakoon *et al.*, 2015). Circulating cathodic antigen is a glycan that is genus specific and is released by adult schistosomes in the blood stream of the definitive host. The glycan circulates in the blood stream of a definitive host, and it can be identified in the urine of an infected person. In diagnosis of *S. mansoni*, a urine sample from a patient is collected and introduced into a POC-CCA kit (dropped into a cassette). The CCA

kit has a labelled monoclonal antibody that is immobilized on the sample membrane. The solution (urine in this case) from the person being screened or diagnosed runs over a strip. In case of a positive case, an antigen-antibody complex is formed. The complex later attaches to a monoclonal antibody that is immobilised at the test line. After 20 minutes of incubation, results can be established by visual reading. Observation of a single line on the kit is an indication that the patient is not infected. On the other hand, observation of double lines on the kit is an indication that the patient is infected (Colley *et al.*, 2013).

Point of Care CCA test has on many occasions been used in the field to establish the prevalence of schistosomiasis as well in the assessment of the success of mass drug administration programmes. Point of Care CCA tests have proven to be more reliable and with higher diagnostic accuracies than Kato Katz tests in field conditions. A study by Adriko *et al.* (2014), evaluated CCA urine- cassette assay in surveillance of *S. mansoni* and found it more satisfactory than the Kato Katz technique (Weerakoon *et al.*, 2015). A study in Kenya by Mwinzi *et al.* (2015) showed that Point of Care CCA test can detect schistosomiasis in areas with low endemicity, where Kato Katz technique yields false negative results.

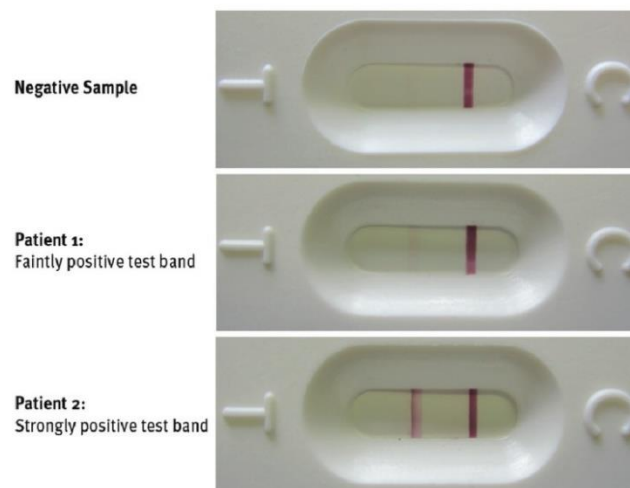


Figure 16: Lateral flow cassette assay (POC-CCA), courtesy of Becker *et al.* (2015).

The urine-CCA dipstick removes the need for faecal sampling because it detects Circulating Cathodic Antigens released by adult schistosomes in a human host's urine. The dipstick can detect schistosome antigens in as little as 15µl volume of urine (Standley *et al.*, 2010). The CCA test has proven to be more effective in detecting *S. mansoni* infection than in detecting *S. haematobium* infection and it is recommended by the World Health Organisation for the screening of intestinal schistosomiasis in endemic areas (Mazigo *et al.*, 2018). The major drawback of CCA dipsticks is the cost, with one dipstick priced at between \$2.3-\$4.6 yet it is not reusable (Standley *et al.*, 2010).

1.4.3 Molecular detection tests

Molecular diagnosis of schistosome infections seems to be the future of schistosomiasis diagnosis. In recent years, there has been the development of molecular diagnostic tests that detect parasite antigens from fluids (urine or blood) of infected human beings and also those that detect specific schistosome DNA sequences in snail tissues. Molecular methods

of schistosome detection, normally involve Polymerase Chain Reaction (PCR) tests. Molecular detection tests function by detecting *Schistosoma* RNA or DNA using PCR (Polymerase Chain Reaction) techniques. The Polymerase Chain Reaction techniques that are used include real-time quantitative PCR or multiplex PCR (Weerakoon *et al.*, 2015). PCR methods are more sensitive and exhibit high specificity compared to parasitological detection tests and immunological detection tests. PCR techniques often amplify a certain segment of a gene, in this case, a Schistosome gene (Weerakoon *et al.*, 2015).

Several PCR tests for the detection of schistosome infection have been developed over the years (Rabello *et al.*, 2002). A real-time PCR technique developed by Hamburger (based on a sequence in the *S. mansoni* genome) showed the ability to detect cercariae at transmission sites (Hamburger *et al.*, 1998a). A PCR technique developed by Sandoval *et al.* (2006) was able to detect schistosome infection in urine samples. The Sandoval technique is genus specific as well as species-specific. The Sandoval assay exhibited a sensitivity and specificity of more than 90% at both genus and species levels. A real-time PCR technique developed by He *et al.* (2018) exhibited a high level of sensitivity in the detection of *S. japonicum* infection in the environment as well as in cattle and bovine stool samples. Based on the sensitivity and specificity displayed by the assay, it can be applied as a surveillance tool in areas characterised by low *S. japonicum* infection transmission rates.

Detection of schistosome infections in snail intermediate hosts is an indirect indicator of the prevalence of schistosomiasis in a certain geographical location. Detection of schistosome infections in intermediate hosts can potentially reveal transmission sites as far as prevalence of schistosomiasis is concerned (Weerakoon *et al.*, 2015). Knowledge obtained by detection of schistosomes in snail intermediate hosts would be important in coming up with effective control programmes as well as bolstering current control programmes (Weerakoon *et al.*, 2015).

DNA amplification methods have over the years been developed to address the limitation of the cercarial shedding method (Weerakoon *et al.*, 2015). DNA amplification methods are more specific and sensitive when compared to cercarial shedding. DNA amplification methods can detect primary sporocysts and secondary sporocysts (prepatent *Schistosoma* parasites stages). In DNA amplification methods, specific gene segments of a particular *Schistosoma* parasites, for instance *S. mansoni* are amplified by first development of primer templates that exhibit the same sequence as the specific gene segment that is to be targeted. A PCR assay method developed by Hamburger *et al.* (1998a) to detect *S. mansoni* from very early prepatency is regarded as the pioneer DNA schistosome detection method in snails. Hanelt *et al.* (1997) developed a PCR protocol that was used to detect *S. mansoni* sporocysts in *Biomphalaria* snails by amplification of 18S rDNA of *S. mansoni* parasite without amplification of 18S sequences of the snail intermediate host.

A PCR technique developed by (Hamburger *et al.*, 1998b) showed the ability to detect larval stages of *Schistosoma* parasites in snail intermediate hosts. The detection of schistosome infection in snail intermediate hosts is likely to reveal more infection than the demonstration of infection by cercarial shedding. A PCR assay developed by Hamburger *et al.* (2004) based on a DRA 1 sequence of *S. haematobium* detected prepatent stages of the parasite from *Bulinus globosus* and patent stages in the outside world from known

transmission sites in the Coastal region of Kenya. A PCR technique developed by Lu *et al.* (2016) based on *S. mansoni*'s ND5 gene detected *S. mansoni* infection in *Biomphalaria* snails. The ND5 PCR technique was sensitive and *S. mansoni* specific. In the assay *B. pfeifferi* showed more compatibility to *S. mansoni* than *B. sudanica*. A study by Joof *et al.* (2020) also confirmed that PCR techniques show a high level of schistosome infection detection by detecting prepatent stages of the parasites in the snail intermediate hosts compared to the level of infection demonstrated by cercarial shedding.

Sandoval *et al.* (2006) developed a PCR method that has been used to diagnose *S. mansoni* infections in human beings using urine samples. Primer pairs were designed with an intention of amplifying *Schistosoma* 28S rDNA region. The Sandoval PCR technique has been used to detect schistosome infection in snail intermediate hosts and has demonstrated high levels of specificity and sensitivity (Joof *et al.*, 2020).

One of the most recent PCR protocols to be developed is one by Lu *et al.* (2016). Lu *et al.* (2016) designed primers in line with *S. mansoni* ND5 gene. It was concluded that the ND5 PCR protocol was species specific, and it was possible for *S. mansoni* to be differentiated from other *Schistosoma* species. The Lu *et al.* (2016) assay is able to detect prepatent *S. mansoni* stages in *Biomphalaria* snails.

However, the PCR tests are yet to be adopted on a wide scale in low resource settings, probably because costs to conduct PCR tests in endemic settings are 'relatively high' and because diagnostic services in endemic settings that mostly double up as low resource settings are not properly established (Rollinson *et al.*, 2009).

1.4.4 Environmental DNA techniques

Ability to detect schistosome infection in the environment (water) is needed for the success of schistosomiasis prevention, control, and elimination programs. Environmental DNA methods provide a novel surveillance approach characterised by rapidity and specificity for the detection of snails and schistosomes in their natural habitats (Kamel *et al.*, 2021). Environmental DNA methods are believed to have the potential to improve schistosomiasis eco-epidemiology studies by rapid identification of safe spots and hotspots in endemic areas without the need of having to identify live parasites and snail hosts in the environment. Environmental DNA methods take away the need to conduct extensive surveys in search of snail intermediate hosts and *Schistosoma* parasites (Sato *et al.*, 2018). The methods work by detecting traces of DNA, also known as environmental DNA, that have been left behind by larval stages of *Schistosoma* parasites as well as traces left by snail intermediate hosts (Sengupta *et al.* 2019; Sato *et al.* 2018). Normally, water samples from areas suspected to be endemic of schistosomiasis are collected and tested using highly specific and sensitive PCR assays (Sato *et al.*, 2018). However, one major drawback of environmental DNA techniques is that they may detect 'old' DNA of schistosomes and snails that are no longer in existence in a particular habitat (providing a false estimation of the infection risk) (Sengupta *et al.*, 2019).

1.5 SNAIL INTERMEDIATE HOSTS

Snail intermediate hosts for *Schistosoma* parasites are mostly aquatic snails and they are important for the completion of *Schistosoma* parasites life cycle because it is in the snails

where development of asexual stages of *Schistosoma* parasites take place. Elimination of snail intermediate hosts from a given ecosystem would interrupt the transmission of schistosomiasis in that locality (Yang *et al.*, 2018).

Snail intermediate hosts differ from one *Schistosoma* species to another. *Biomphalaria* snails are the principal snail intermediate hosts for *S. mansoni* with *B. glabrata* being the most important snail intermediate host for *S. mansoni* in the Americas and *B. pfeifferi* in Africa (because of its widespread geographical distribution) (Yang *et al.*, 2018). *Bulinus* snails are the principal snail intermediate hosts for *S. haematobium*, *S. intercalatum* and *S. guineensis*. *Bulinus* snails include *B. truncatas*, *B. africanus*, *B. globosus*, *B. forskalii*, *B. camerunensis*, *B. tropicus* and *B. natalensis*. *Oncomelania* snails are the only intermediate hosts for *S. japonicum*. The snails are highly invasive and are primarily found in Asia. In addition, *Oncomelania* snails are amphibious, meaning that they can survive both on land and in water. The ability of *Oncomelania* snails to live in both water and land makes them the perfect snail intermediate hosts for *Schistosoma* parasites because they are able to withstand seasonality changes, something that other snail intermediate hosts struggle with because they can only survive in aquatic environments with the right water volume (Zhou *et al.*, 2020). *Neotricula* snails serve as the snail intermediate hosts for *S. mekongi*, a parasite restricted in distribution to the Mekong River region in Southeast Asia (Yang *et al.*, 2018).

Climatic and environmental factors contribute towards creating suitable environments for the habitation of snail intermediate hosts. In addition to climatic and environmental factors, human activities also play a role in promoting the abundance and distribution of the snail intermediate hosts. Establishment of irrigation schemes, canals and hydroelectric dams continue to create new habitats that are suitable for the habitation of the snail intermediate hosts (Yang *et al.*, 2018).

There is evidence that asserts that the trade in aquarium plants has introduced *Biomphalaria* and *Bulinus* snails in North America and Europe, thus making it possible for the life cycle of *Schistosoma* parasites to continue in those geographical areas (new territories) (Majoros *et al.*, 2008). In addition, increase in global temperatures courtesy of climate change has made water bodies in Europe and North America, more suitable for the habitation of *Schistosoma* parasites' snail intermediate hosts. Consequentially, *Schistosoma* parasites have been able to conquer new snail intermediate hosts in the new territories of North America and Europe, thus increasing their geographical range (Kincaid-Smith *et al.*, 2017). *Biomphalaria* snails maintain a narrow thermal niche and are rarely found in higher latitudes, except for the case of *Biomphalaria teganophila* that was found in Romania. On the other hand, *Bulinus* snails seem to possess a relatively broader thermal niche with *Bulinus truncatas* having been found in Corsica, a Mediterranean island, located between France and Italy (Mulero *et al.*, 2019). This therefore means that *S. haematobium* introduced in the Mediterranean region by immigrants are likely to find their ideal snail intermediate host and therefore continue their asexual life cycle normally. The most famous atypical snail intermediate hosts of *Schistosoma* parasites in Europe are *Planorbis metidjensis*, *Lymnaea stagnalis* and *Radix natalensis* (Kincaid-Smith *et al.*, 2017).

1.6 BIOMPHALARIA SNAILS

1.6.1 Distribution of *Biomphalaria* snails

Biomphalaria snails are widely distributed across Africa, South America, and Asia. In the Americas, the snails are found in several Caribbean islands, including Guadeloupe, Puerto Rico, the Dominican Republic, and St. Lucia. In the South American continent, the snails are found in Venezuela, Suriname, and Brazil. In Africa, the snails are extensively distributed across the continent. The snails are also distributed in parts of the Arabian Peninsula and Southeast Asia, notably Hong Kong where *Biomphalaria straminea* is common (Toledo & Fried, 2011).

Biomphalaria snails inhabit all types of freshwater bodies in the tropics and subtropics (Toledo & Fried, 2011). The freshwater bodies include lakes, rivers, streams, and temporary ponds. Distribution of the snails in the water bodies is not always even. As a matter of fact, most of the time, the distribution of *Biomphalaria* snails in water bodies is patchy. Interestingly, the snails have a predilection for shallow waters in streams, irrigation channels, ponds, and shores of lakes than deep waters in rivers and lakes. *Biomphalaria* snails also have a penchant for living close to vegetation found at the banks of rivers, in streams and in lakeshores. It has been established that plants play a major role in helping *Biomphalaria* snails thrive in their habitats. Plants protect *Biomphalaria* snails from being washed away by high water velocities as well as by serving as substrates for oviposition and feeding. (Toledo & Fried, 2011).

In addition, the snails also prefer habitats rich in mud that is rich in decaying matter. The snails would rather inhabit water bodies that are rich in organic matter than water bodies that are not rich in organic matter. Richness in organic matter is a major reason why *Biomphalaria* snails inhabit habitats close to where human beings engage in activities such as defaecation and urination. Urine and faeces from human beings are rich in organic matter (Toledo & Fried, 2011).

Biomphalaria snails normally burrow themselves in mud substratum to avoid coming in contact with high temperatures as well as avoid being swept away by fast flowing waters. Snails that burrow themselves in mud survive dry periods (McCreesh *et al.*, 2014). Drought is very detrimental to the survival of *Biomphalaria* snails because it often annihilates a large number of *Biomphalaria* snails (Toledo & Fried, 2011). Abnormal temperatures may have both stimulatory and inhibitory effects on *Biomphalaria* snails' immune defence system response to schistosome infections (Nelson *et al.*, 2016).

Biomphalaria snails prefer water that is rich in Ca²⁺ (Brown, 1994). This has been attributed to the importance of Ca²⁺ in the development of snail shells (Brown, 1994). A study by Brodersen & Madsen (2003) discovered a direct relationship between juvenile snails' shell size and Ca²⁺ concentration in waters inhabited by the snails. It was also established that there is a direct relationship between shell strength and the concentration of Ca²⁺ in the waters inhabited by the snails. Shells of snails that inhabit high concentration of Ca²⁺ crash with more difficulty when compared to those of snails that inhabit waters with a low concentration of Ca²⁺. It therefore indicates that *Biomphalaria* snails are less likely to survive in waters with low Ca²⁺ concentrations (Brodersen & Madsen, 2003).

1.6.2 Classification and evolutionary history

In general, there are 34 species of Genus *Biomphalaria*. Out of the 34 species, 22 are found in the 'New World' and 12 are found in the 'Old World.' *Biomphalaria* snails that are found in the 'New World' are: *Biomphalaria glabrata*, *Biomphalaria teganophila*, *Biomphalaria amazonica*, *Biomphalaria straminea*, *Biomphalaria intermedia*, *Biomphalaria andecola*, *Biomphalaria havanensis*, *Biomphalaria oligoza*, *Biomphalaria prona*, *Biomphalaria obstructa*, *Biomphalaria edisoni*, *Biomphalaria manya*, *Biomphalaria schrammi*, *Biomphalaria straminea*, *Biomphalaria temascalensis*, *Biomphalaria subprona*, *Biomphalaria occidentalis*, *Biomphalaria orbigny*, *Biomphalaria schrammi*, *Biomphalaria manya* and *Biomphalaria kubiana* (Malek, 1985). *Biomphalaria* snails that are found in the Old World include *Biomphalaria smithi*, *Biomphalaria alexandrina*, *Biomphalaria pfeifferi*, *Biomphalaria angulosa*, *Biomphalaria choanomphala*, *Biomphalaria sudanica*, *Biomphalaria stanleyi*, *Biomphalaria salinarium*, *Biomphalaria camerunensis*, *Biomphalaria ruppellii*, *Biomphalaria barthi* and *Biomphalaria tchadiensis*. However, it is worthy to mention that *B. straminea* is found both in South America and in Asia (Toledo & Fried, 2011).

Classification of *Biomphalaria* snails has traditionally been based on shell characters. Shells of *Biomphalaria* snails generally have a diameter of 10mm to 40mm with *B. glabrata* snails having the largest shells with a diameter of 40 mm. Shells of *Biomphalaria* snails are generally coiled and sinistral (Figure 17) (Toledo & Fried, 2011). There are two general clades of *Biomphalaria* snails; one is the Neotropical clade and the other is the African clade, with the Neotropical clade consisting of *Biomphalaria teganophila* and *Biomphalaria straminea* (Jorgensen *et al.*, 2007). The genetic distance that exists between African *Biomphalaria* species is smaller compared to one that exists between Neotropical species affirming a recent origin of the African species (Campbell *et al.*, 2000). According to DeJong *et al.* (2001), all African *Biomphalaria* species arose from a proto- *Biomphalaria glabrata* snail through a transatlantic colonization of Africa, tentatively 2.5 MYA. Table 1 shows African *Biomphalaria* species and the location where each particular species has previously been found, courtesy of Jorgensen *et al.* (2007).

<i>Biomphalaria</i> species	Country/location found
<i>Biomphalaria glabrata</i>	Egypt
<i>Biomphalaria camerunensis</i>	Cameroon
<i>Biomphalaria pfeifferi</i>	Sudan, Senegal, Zimbabwe, Kenya
<i>Biomphalaria angulosa</i>	Tanzania
<i>Biomphalaria choanomphala</i>	Lake Victoria (Uganda)
<i>Biomphalaria stanleyi</i>	Lake Albert (Uganda)
<i>Biomphalaria smithi</i>	Lake Edward (Uganda)
<i>Biomphalaria alexandrina</i>	Egypt
<i>"Biomphalaria pfeifferi"/</i> <i>Biomphalaria</i> sp.	Masindi Port, Lwampanga, Ngamilajojo, Muzizi (Uganda)
<i>Biomphalaria sudanica</i>	Rutoto, Ntoroko (Uganda)
<i>Biomphalaria cf. pfeifferi</i>	Lake Albert (Uganda)
<i>Biomphalaria sudanica</i>	Kianyara, Lake Albert, Mahyoro

Table 1: A Summary Table of African *Biomphalaria* species courtesy of Jorgensen *et al.* (2007).

There are two general lineages of African *Biomphalaria* snails. One lineage is the *B. pfeifferi*/*Biomphalaria camerunensis* lineage and the other lineage is the *Biomphalaria angulosa*/Nilotic species complex lineage (Jorgensen *et al.*, 2007). After the initial colonisation of Africa, *Biomphalaria* snails migrated to the East. In East Africa, there was further dispersal of the snails in the Rift Valley lakes and in the Lake Victoria basin. Subsequent dispersal along the River Nile led to additional speciation and emergence of *Biomphalaria alexandrina* (Toledo & Fried, 2011).

The 'Nilotic species complex' was formed after a *B. pfeifferi* type of snail migrated to East Africa, followed by speciation, leading to the formation of *B. angulosa*. With *B. angulosa*, acting as the basal node, the 'Nilotic species complex' was formed. The 'Nilotic species complex' consists of *B. alexandrina*, *B. choanomphala*, *B. sudanica*, *B. stanleyi* and *B. smithi*. The Nilotic species members are distributed majorly in water bodies that are close to River Nile (DeJong *et al.*, 2001).

A study by Bandoni *et al.* (2000) in an attempt to classify African *Biomphalaria* species using allozymic markers was a pioneer study in the molecular identification of African *Biomphalaria* snails. The genetic basis for three African *Biomphalaria* species (*B. choanomphala*, *B. sudanica* and *B. pfeifferi* from Kenya) was examined and it was

concluded that populations of *B. choanomphala* showed the least evidence of inbreeding whereas populations of *B. pfeifferi* showed the highest evidence of inbreeding, with *B. sudanica* being intermediate.



Figure 17: Sinistral coiling of *Biomphalaria* snails' shells.

Biomphalaria snails in Africa have been categorised into four species groups (Mandahl-Barth, 1957). These species groups are the *B. pfeifferi* group, the *B. choanomphala* group, the *B. alexandrina* group and the *B. sudanica* group. The *B. pfeifferi* group consists of *Biomphalaria pfeifferi* and *Biomphalaria rhodesiensis*. The *B. choanomphala* group consists of *Biomphalaria barthi*, *Biomphalaria choanomphala*, *Biomphalaria smithi* and *Biomphalaria stanleyi*. The *B. alexandrina* group consists of *Biomphalaria alexandrina*, *Biomphalaria angulosa*, *Biomphalaria salinarium* and *Biomphalaria tchadiensis*. The *B. sudanica* group consists of *Biomphalaria camerunensis* and *Biomphalaria sudanica* (Campbell *et al.*, 2000). However, molecular data from a study by Jorgensen *et al.* (2007) disputes the categorisation of *B. camerunensis* as a member of the *B. sudanica* group.

DeJong *et al.* (2003) reckon that the oldest haplotypes of *B. pfeifferi* occur in Southern Africa and that recent haplotypes of *B. pfeifferi* snails are found in East Africa. It has further been established that several species and sub-species of *Biomphalaria* snails in East Africa and the Middle East have little genetic differentiation from *B. pfeifferi*. The species and sub-species that have little genetic differentiation from *B. pfeifferi* include *Biomphalaria stanleyi*, *Biomphalaria arabica*, *Biomphalaria rhodesiensis* and *Biomphalaria gaudi* (DeJong *et al.*, 2003).

Cox1 variation within and between *Biomphalaria* snails in Africa versus America has been ascertained by Zhou *et al.* (2019), Zhang *et al.* (2018) & Standley *et al.* (2011). A mitochondrial Cox1 analysis of African and American *Biomphalaria* snails discovered that the identity between *B. choanomphala* and *B. sudanica* at the nucleotide level was 98.31%. On the other hand, the identity of two strains of *B. glabrata* at the nucleotide level was 94.95%. *B. tenagophila* was revealed to have the lowest identity with *B. choanomphala*, *B. sudanica* and *B. glabrata* (85.31-86.06% at the nucleotide level) (Zhang *et al.*, 2018). Phylogenetic analysis of 12 protein coding mitochondrial genes of *B. straminea* snails found in China revealed that *B. straminea* snails (which are found in both Asia and South America) are closely related to *B. glabrata* than *B. tenagophila* (Zhou *et al.*, 2019). A mitochondrial Cox1

analysis of *Biomphalaria* snails collected from Lake Victoria by Standley *et al.* (2011) proposed *B. choanomphala* and *B. sudanica* be considered as two subspecies.

There has been contention among taxonomists on the classification of different African *Biomphalaria* species. Use of shell morphology alone in identification of African *Biomphalaria* snails has proved elusive. The identity of *B. choanomphala* and *B. sudanica* as separate species has been disputed by Standley *et al.* (2011) who with the use of morphological and molecular variables demonstrated inconsistencies between morphological divisions and molecular groupings. Phenotypic plasticity, more so among closely related snail types has been shown to be environmentally determined (Urabe, 1998). A study by Jorgensen *et al.* (2007) discovered snails with *B. pfeifferi* like morphology that actually belonged to the Nilotic species complex when genetic analysis was conducted. Subsequently, a *B. pfeifferi* snail found at Lake Albert was initially thought to be *B. stanleyi* until molecular analysis was conducted, thus suggesting that *B. stanleyi* could be an ecophenotypic form of *B. pfeifferi* adapted to a lentic (lacustrine) environment (Plam *et al.*, 2008). Standley *et al.* (2011) affirms that habitat has a significant impact on the morphology of *Biomphalaria* snails and thus morphology alone is not reliable in the identification of the snails and that the two forms of *Biomphalaria* found in Lake Victoria are ecophenotypes of the same species. Standley *et al.* (2011) suggests that rather than being called *B. choanomphala* and *B. sudanica*, the two forms of *Biomphalaria* found in Lake Victoria should be called *B. choanomphala choanomphala* and *B. choanomphala sudanica* respectively. Suffice is to say that phenotypic plasticity and the development of ecotypes makes it difficult to differentiate *Biomphalaria* snails solely on shell morphometrics (Standley *et al.*, 2011).

1.6.3 *Biomphalaria pfeifferi*

Biomphalaria pfeifferi is arguably the most important snail intermediate host in the transmission of schistosomiasis because of its widespread distribution. *B. pfeifferi* snails are primarily found in sub-Saharan Africa and in a few parts of the Middle East. Morphologically, *B. pfeifferi* snails have a shell diameter of about 15 mm with a maximum of five whorls, which are either convex or angular, and the diameter of the umbilicus is about 3.0mm (Mandahl, 1957).

The snails prefer water bodies that are shallow, with low water volumes and slow flowing (of speeds less than 0.2m/s) (Utzinger & Tanner, 2000; Utzinger *et al.*, 1997; Babiker *et al.*, 1984). Fast flowing waters have been linked to the death of *B. pfeifferi* snails, more so among juvenile snails. Entire snail populations in erstwhile slow flowing water bodies are likely to be destroyed during heavy rains. Furthermore, the snails prefer edges of shallow water bodies, and this probably explains why *B. pfeifferi* snails have been found in rivers, ponds, irrigation schemes and streams, but not in deep lakes (Boelee & Madsen, 2006). In addition, *B. pfeifferi* snails have a preference for rivers, irrigation canals and streams that have dense aquatic vegetation because the vegetation is food for the snails and because the vegetation prevents the snails from being swept away by high water currents (Utzinger *et al.*, 1997). *B. pfeifferi* snails have on many occasions been found in irrigation schemes with high densities being found mostly upstream, where the flow of water is relatively calm (Mutuku *et al.*, 2014; Boelee & Madsen, 2006; Babiker *et al.*, 1984). Irrigation canals that are

less than 10 meters wide have also been found suitable for the habitation of *B. pfeifferi* snails (Boelee & Madsen, 2006). It has also been established that *B. pfeifferi* snails prefer muddy soil because the stickiness of muddy soils prevents them from being washed away by fast flowing waters (Boelee & Madsen, 2006).

B. pfeifferi snails are cold-blooded animals and therefore, outside temperature is very crucial as far as their survival is concerned (Nelson *et al.*, 2016). Unfavourable temperatures suppress the geographical distribution of the snails (Appleton & Eriksson 1984). *B. pfeifferi* snails die when temperatures become very low, below freezing point and when they become high, above 27°C. Furthermore, production of eggs as well as gonad development is impaired by high temperatures, above 27 degrees Celsius (Appleton & Eriksson 1984).

Organic matter has been established to play a role in the geographical distribution of *B. pfeifferi*. *B. pfeifferi* snails thrive in water bodies that have high concentrations of organic matter because the snails feed on decaying organic matter (Klutse & Baleux, 1996). *B. pfeifferi* snails have also been found in water bodies contaminated with sewage and faecal matter (Kloos *et al.*, 2004).

The role of pH in the geographical distribution of *B. pfeifferi* snails has been found to be insignificant. It has been proven that pH has little effect on the distribution of *B. pfeifferi* snails (Kengne-Fokam *et al.*, 2016). Furthermore, it has been ascertained that gastropods are able to tolerate different pH values.

According to Lu *et al.* (2016), compatibility of *B. pfeifferi* with *S. mansoni* is relatively higher than compatibility of *S. mansoni* with other *Biomphalaria* species. The relative higher compatibility and widespread geographical distribution are the reasons *B. pfeifferi* is arguably the most important intermediate host in the transmission of human intestinal schistosomiasis (Lu *et al.*, 2016).

High self-fertilization rates have been reported in many *B. pfeifferi* populations (Charbonnel *et al.*, 2002a) and (Charbonnel *et al.*, 2002b). Self-fertilization as a preferential mating system often comes in handy when the population size of pulmonate snails has shrunk, courtesy of seasonal changes and other stressors (Kengne-Fokam *et al.*, 2016). However, survival rates in snails where fertilization takes place by cross-fertilization is higher than in those where fertilization has taken place by self-fertilization with the time it takes for *B. pfeifferi* snails to reach maturity being approximately 63 days after hatching of eggs has taken place (Kengne-Fokam *et al.*, 2016).

Distribution of *B. pfeifferi* snails is affected by biotic and abiotic factors and this include water availability, water depth, water flow, water velocity as well as calcium availability (Jarne & Delay, 1991). *B. pfeifferi* is generally non-lacustrine. It prefers habitats whose water availability varies seasonally. The variation in water availability seasonally induces fluctuation of the snails' population density. Reduction in population size together with possible extinction of *Biomphalaria* snails can be caused by drought. However, *B. pfeifferi* snails are able to survive during dry periods by hiding in mud (Jarne & Delay, 1991).

1.6.4 *Biomphalaria choanomphala*

Biomphalaria choanomphala is a member of the 'Nilotic species complex' and it has been found in Lake Victoria and Lake Albert (Mutuku *et al.*, 2021; Gouvras *et al.*, 2017; Rowel *et al.* 2015; Standley *et al.* 2014 & Magendantz, 1972). Divergence of opinion among taxonomists on *Biomphalaria* snails that were erstwhile regarded as *B. sudanica* suggest that the former should be regarded as a subspecies of *B. choanomphala*. Therefore, in this classification, we describe snails formerly known as *B. chonomphala* before 2007 as *B. choanomphala choanomphala* or *B. choanomphala* type and those formerly known as *B. sudanica* as *B. chonomphala sudanica* or *B. sudanica* type (Standley *et al.*, 2011).

B. choanomphala type is highly associated with Lake Victoria's deeper waters (Gouvras *et al.* 2017; Rowel *et al.* 2015; Standley *et al.* 2014 & Magendantz, 1972). *B. choanomphala* type has been found in the lake bottom of Lake Victoria, up to 150 m offshore. *B. choanomphala* type is largely regarded as a lacustrine species, having been found by several studies in the lake proper (greater depth) of Lake Victoria. Morphologically, *B. choanomphala* type is relatively smaller in size when compared to *B. pfeifferi* and *B. sudanica* type. *B. choanomphala* type shells have a diameter of less than 12 mm and a height of about 3.4 mm, with more angular whorls (Mandahl-Barth, 1957).

High snail densities of *B. choanomphala* type in Lake Victoria have been found on mixed sand/ mud substrata, rich in algal growth and containing a moderate amount of organic matter (Magendantz, 1972). A high calcium content in the lake waters has also been established to be influential in the distribution of *B. choanomphala* type snails (Magendantz, 1972).

Naturally occurring *S. mansoni* infection rates in *B. choanomphala* type snails have been found to be associated with the age of the snails, with higher infection rates being found in older snails. The infection rates have also been found to be associated with snail densities, with infection rates higher in low snail densities (Magendantz, 1972). A study by Mutuku *et al.* (2021) asserts that *B. choanomphala* type is more compatible with *S. mansoni* than *B. sudanica* type. In Ugandan Lake Victoria, *B. choanomphala* type is regarded as the most important transmitter of *S. mansoni* because more naturally *S. mansoni* infected *B. choanomphala* type populations have been found than naturally infected *B. sudanica* populations (Standley *et al.*, 2011).

B. sudanica type has severally been found in emergent vegetation next to Lake Victoria (Gouvras *et al.* 2017; Magendantz, 1972). Morphologically, *B. sudanica* type snails have a large shell of a diameter that is up to 22 mm (Mandahl, 1957). The shells have about five to six whorls that are flat or somehow angular with the diameter of the shell being at least three times the height of the last whorl.

Snail densities of *B. sudanica* type around Lake Victoria have been associated with seasonality with the highest number of *B. sudanica* type snails being found during the dry season and the lowest number being found during the rainy season. On the other hand, factors such as pH and dissolved ions, potentially have no discernible effect on *B. sudanica* type snail densities (Gouvras *et al.*, 2017).

S. mansoni infection rates in *B. sudanica* type populations found in Lake Victoria have been associated with the proximity of the snails to open shores. The proximity of *B. sudanica* type populations to open shores together with activities such as fishing, washing, bathing and pollution play an important role in contributing to high schistosome infection rates in the *B. sudanica* type snails. This is because active miracidia from schistosome eggs deposited in the environment by infected individuals can easily find their way to the intermediate hosts (Magendantz, 1972).

1.6.5 *Biomphalaria smithi*

Biomphalaria smithi is a member of the 'Nilotic species complex' of *Biomphalaria* snails. The snail has a shell with a width that ranges from 7.4 cm to 9.6 cm (Brown, 1994). It has been revealed that *B. smithi* has morphological characteristics that are almost similar to those of *B. choanomphala* (Plam *et al.*, 2008). However, *B. smithi*'s strongly downward sloping body whorl distinguishes it from the other *Biomphalaria* snails (Plam *et al.*, 2008).

B. smithi is endemic to Lake Edward, Uganda where it has an affinity for *Vallisneria* aquatic plants, but it has also been found in Lake Albert (Plam *et al.* 2008; DeJong *et al.*, 2001).

1.6.6 *Biomphalaria stanleyi*

Biomphalaria stanleyi has been found in Lake Albert, Uganda and according to DeJong *et al.* (2001), the snail is a lacustrine ecophenotype of *B. pfeifferi*. Brown, (1994) found *B. stanleyi* in Lake Chad and in Lake Cohoha in Burundi. A study by Plam *et al.* (2008) suggests that *B. stanleyi* is likely to be an ecophenotype of *B. pfeifferi* in a lacustrine environment.

Morphologically, *B. stanleyi* shell is of small to medium in size (with a mean diameter of about 10mm); and relatively raised when compared to any other *Biomphalaria* species. The shell also consists of less than four whorls that are more high than wide (Kristensen, 1987).

1.6.7 *Biomphalaria angulosa*

Biomphalaria angulosa is regarded as a sister group to the 'Nilotic species complex'. Morphologically, *B. angulosa*'s shell has a closer resemblance to *B. camerunensis*' shell. The shell is large, with a mean diameter of 15.00mm and consists of 4 to 5 whorls that are angular (Mandahl, 1957). *B. angulosa*'s reddish colour is a distinctive feature that separates it from the other African *Biomphalaria* snails.

According to phylogenetic studies, *B. angulosa* takes a basal position to the 'Nilotic species complex' (Jorgensen *et al.*, 2007). It is worthy to mention that *B. angulosa* is not commonly found, having only been found in a few locations in Southern Tanzania and Northern Zambia (Kristensen, 1987).

1.7 POPULATION GENETICS STUDIES ON *BIOMPHALARIA* SNAILS

Population genetic structure refers to the distribution of alleles at different loci within and among populations (Jarne & Delay, 1991). Population genetic studies aim to explain the distribution of genetic diversity within and among populations (Mavarez *et al.*, 2002). Genetic variations within and among populations is caused by a concert of factors (Wethington *et al.*, 2007). Genetic drift, migration, mating, gene flow, parasitism, mutation, and natural selection are some of the factors that cause genetic variations within and

among populations. Environmental factors such as flooding and droughts also affect the population genetic structure of *Biomphalaria* snails with Analysis of Molecular Variance used to test genetic differentiation between and within populations (Thiele *et al.*, 2013).

F_{ST} value is a measure of genetic distance between populations. The greater the genetic distance between populations, the more isolated they are from each other and the less breeding that happens between them. The lower the genetic distance between populations, the less isolated they are from each other and the more breeding that takes place between them. F_{ST} values can range from 0 to 1, where 0 means that there is complete sharing of genetic material, whereas 1 means that there is no sharing of genetic material. F_{ST} value can also be defined as the measure of the difference in allele frequency between two populations. F_{ST} values are important in population genetic studies because they indicate the levels of connectivity between populations. Low F_{ST} values means high connectivity between populations or low genetic differentiation between populations. High F_{ST} values on the other hand mean low connectivity between populations or high genetic differentiation between populations. F_{ST} value is more than a measure of genetic differentiation because it is directly related to the variance in allele frequency among populations and the genetic resemblance among individuals within populations. When an F_{ST} value is small, it implies that the allele frequency within each population is similar whereas on the other hand when F_{ST} value is large, it means that allele frequencies within the populations are different (Holsinger & Weir, 2019). The use of F_{ST} values in this study is justified because we would like to understand the genetic differences between *Biomphalaria* populations.

AMOVA (Analysis of Molecular Variance) is used to calculate the level of molecular variation among different populations within a single species (Excoffier *et al.*, 1992). The use of AMOVA in this study is justified because we intend to evaluate the genetic differentiation that exists between the different *Biomphalaria* populations.

High genetic diversity in *Biomphalaria* populations is often associated with random mating and high gene flow. Restricted or spatial gene flow leads to low genetic diversity. Migration plays a role in modification of allele frequencies among populations. Without migration and gene flow, there would be an increase in genetic variation and possible speciation (Thiele *et al.*, 2013). A study by Thiele *et al.* (2013) on *Biomphalaria* populations in Brazilian villages revealed that most subpopulations exhibited relatively low genetic diversity, manifested by heterozygote deficits. Genetic diversity in the villages was spatial among *B. glabrata* populations.

The more genetically diverse *Biomphalaria* snail populations are, the less compatible they are with schistosomes. The more genetically diverse, *Biomphalaria* populations are, the less parasite transmission rates are successful in them (Kengne-Fokam *et al.*, 2016). *B. pfeifferi* is notable for engaging in self-fertilization and being strongly affected by bottlenecks such as droughts and floods. Consequentially, this often leads to limited genetic diversity within populations but a higher genetic differentiation between populations (Mutsaka *et al.*, 2020). Genetic variability in *B. alexandrina* snails have been associated with differences in *S. mansoni* infection rates (Azza *et al.*, 2012).

Geographical distance also has an influence on the genetic differentiation that exists between populations. Individuals from sites that are closer to each other are likely to be genetically similar whereas individuals from sites that are geographically distant are likely to have a high level of genetic differentiation (Standley *et al.*, 2014). A study by Webster *et al.* (2001a) revealed that levels of intrapopulation genetic similarity were high during drought and lowest following heavy rainfall; this is because during drought the snails became static while after heavy rainfall, the snails were able to move around (migrate) and engage in random mating, facilitating gene flow. A study by Hoffman *et al.* (1998) of 15 *B. pfeifferi* populations on a 6 km stretch of a Zimbabwean river revealed extensive genetic differentiation of the populations, even those being geographically close as 100m were found to be genetically distinct. Members of the 'Nilotic Species Complex' are known to inhabit permanent water bodies such as lakes and rivers that allow migration and facilitate gene flow. As a result, incidents of cross fertilization are more common among the 'Nilotic Species Complex' leading to increase in heterozygosity in populations and reduced interpopulation genetic variation (Standley *et al.*, 2014). This implies that generally, *B. pfeifferi* populations exhibit higher levels of interpopulation genetic variation when compared to members of the 'Nilotic Species Complex.'

1.7.1 Genetic drift

Genetic drift refers to a process whereby changes in allele frequencies occur over generations, in a random manner (Wethington *et al.*, 2007). Effects of genetic drift are stronger in small populations than in large populations. Genetic drift may lead to the loss of alleles as well as an increase in the frequency of certain alleles. Genetic drift is a factor that has a great impact on the genetic structure of any given population. Bottleneck effect and founder effect are the two mechanisms that govern genetic drift. High population differentiation is associated with genetic drift (Wethington *et al.*, 2007).

Bottleneck effect refers to a situation whereby a disaster happens in a population, leading to a reduction in population size and leaving behind few members of the population who do not represent the actual genetic makeup of the original population (Fitzpatrick & Reid, 2019). The few members of the population who survive the disaster often exhibit few genetic variations, amongst themselves when compared to members of the original population. Bottleneck effect leads to less genetic variation among subsequent generations of any given population. Environmental changes are likely to expose snail populations to a number of stressors, some of them maladaptive, subsequently leading to the decline in population size (Fitzpatrick & Reid, 2019). Environmental changes may also lead to extirpation of snail populations. Environmental stressors may also constrain small snail populations that have a high genetic load from withstanding environmental stress (Fitzpatrick & Reid, 2019). Bottleneck effect factors that affect *Biomphalaria* populations include drought, flooding and molluscicides. It has been established that bottlenecks promote high selfing rates in *Biomphalaria* populations. In addition, bottlenecks severely depress the genetic diversity within *Biomphalaria* populations. Natural *Biomphalaria* populations that have for a long time been isolated are subjected to extreme stress and reduced genetic variation makes them less stress tolerant (Charbonnel *et al.*, 2002b).

Founder effect refers to a genetic drift mechanism that leads to the loss in variation of alleles after a new population drifts away from the source population. In 'founder effect', a

small group of a population splits from the main population, subsequently finding its colony. The new colony is definitely isolated from the source population and the founding members of the new colony are likely not to represent the genetic diversity of the source population. Alleles in the new colony may be present in the original population but at different frequencies. At the same time, some alleles in the source population may be missing in the new population. Loss of alleles because of geographical isolation cumulatively leads to less considerable genetic variation in the 'founder population.' Inbreeding is common among 'founder' populations of *Biomphalaria* snails, promoting homozygosity (Wethington *et al.*, 2007).

1.7.2 Migration

Migration plays an important role in increasing genetic diversity among invertebrates (Charbonnel *et al.*, 2002b). Among *Biomphalaria* snails, it has been established that there is frequent migration of snails that inhabit rivers and limited migration events in snails that inhabit watersheds (Charbonnel *et al.*, 2002b). As a result, there is genetic differentiation among snails that inhabit rivers and those that inhabit watersheds. Snail migration often occurs at short distances, following or against water flow. However, dispersal of snails is also possible by animals (such as water birds) and by human activities (Charbonnel *et al.*, 2002a).

Habitat opening has been established to have an effect on the genetic structure of *Biomphalaria* snails in any given habitat (Charbonnel *et al.*, 2002a). Open habitats in this context include rivers whereas closed habitats include canals and ponds. Canals and ponds are more likely to be affected by drought when compared to rivers. Less genetic diversity is expected more in closed habitats than in open habitats. Less genetic diversity is observed more in snails that inhabit seasonal rivers than those that inhabit permanent rivers (Tian-Bi *et al.*, 2013). It has also been established that there is a direct connection between temporary habitats and genetic drift. Snail populations that occupy network of ponds tend to act more as finite 'islands' than as meta-populations (Charbonnel *et al.*, 2002b).

Genetic analysis has revealed that migration in *Biomphalaria* snails often occurs among populations that are geographically close (Bandoni *et al.*, 1990). Studies have revealed that gene flow among *Schistosoma* parasites is high whereas gene flow among most *Biomphalaria* populations is spatially restricted. Constant movement and migration of definitive hosts (mainly man) has been established to be a major reason why gene flow in *Schistosoma* parasites is high (Thiele *et al.*, 2013).

Immigration rates in *Biomphalaria* snails have a ripple effect in that they discourage the snail populations from engaging in self-fertilisation. Self-fertilization in *Biomphalaria* snails reduces with immigration rates and increases with extinction rates (Charbonnel *et al.*, 2002). During the dry season, high rates of self-fertilization are expected due to high chances of genetic drift in snails' populations. Migration depresses the effect that genetic drift has on small snail populations, leading to an increase in genetic diversity in small populations. Aestivation of *Biomphalaria* snails in mud during dry periods also increases genetic differentiation among *Biomphalaria* populations (Tian-Bi *et al.*, 2013).

1.7.3 Mating

Intra-population genetic diversity in bisexual individuals is often higher than in populations where individuals are parthenogenetic (Jarne & Delay, 1991). The mating systems of freshwater snails influence the genetic structure of their populations (Puurtilinen *et al.*, 2004; Azza *et al.*, 2012). Inbreeding in populations leads to allele frequencies in those very populations to remain constant. On the other hand, founder effects cause high genetic variability between populations and at the same time lead to a deficit of heterozygotes within populations. In situations where outbreeding occurs, heterozygote alleles are often found in plenty with it being asserted that random mating promotes heterozygosity (Charbonnel *et al.*, 2002a).

Self-fertilization in *Biomphalaria* snails reduces genetic diversity within snail populations and at the same time increases genetic differentiation among populations (Charbonnel *et al.*, 2002a). Low numbers of alleles are expected in small populations with high selfing rates; subsequently leading to low mean gene diversity. Furthermore, self-fertilization promotes inbreeding depression, which is the reduction in the biological fitness of individuals in a population and it is characterised by high levels of homozygosity. High selfing rates are mainly caused by a decrease in population size as well as reduced migration rates (Charbonnel *et al.*, 2002b).

1.7.4 Gene flow

Gene flow refers to the movement and incorporation of alleles among populations (Jarne & Delay, 1991). Gene flow in *Biomphalaria* snails is promoted by transportation of eggs of snails by birds as well as by passive dispersal during floods. Gene flow is a force that prevents high genetic differentiation between populations. High population differentiation generally means low gene flow. Gene flow is likely to play a major role in the introduction of adaptive alleles as well as amelioration of genetic constraints associated with inbreeding depression (Jarne & Delay, 1991).

Migration is one of the key factors that facilitate gene flow. Migration normally introduces new genes into or out a population. When individuals migrate and reproduce in their new populations, they introduce new genes and gametes into that very population. Gene flow can lead to genetic homogeneity within a metapopulation, in the absence of genetic drift and natural selection. On the other hand, restricted gene flow does promote population divergence which in extreme cases, can lead to speciation (Thiele *et al.*, 2013).

Understanding gene flow patterns in *Biomphalaria* snails is very important because this can help in making predictions on the transmission of schistosomiasis in any geographical area (Wethington *et al.*, 2007). Low gene flow among *Biomphalaria* populations is likely to be the cause of low genetic diversity in any given population with lack of interconnected waterways playing an important role in limiting gene flow between different *Biomphalaria* populations (Vidigal *et al.*, 1998).

1.7.5 Parasitism and population genetics

Parasites can act as extrinsic factors with the potential to severely affect the population genetic structure of snail intermediate hosts by promoting intrapopulation genetic diversity

and interpopulation genetic diversity among *Biomphalaria* snails (Standley *et al.* 2014). Sympatric parasite pressure has the likelihood to promote genetic structuring of subdivided populations (Webster *et al.*, 2001). Parasitic trematodes have been reported to promote mortality in snail populations and at the same time reduce reproduction rates and general physical fitness in those snails (Standley *et al.* 2014). Parasitism has also been shown to have an influence on the fertility of *Biomphalaria* snails. *Biomphalaria* snails that are resistant to schistosome infections have been shown to exhibit reduced offspring production (Webster & Woolhouse, 1999). It has also been established that parasites discourage outcrossing and promote self-fertilisation in snail populations because selfing leads to homozygosity and homozygosity promotes the success of parasitic trematodes infection rates (Jarne & Delay, 1991).

It has also been established that a relationship exists between heterozygosity of *Biomphalaria* snails and *S. mansoni* infection status (Standley *et al.*, 2014; Sandland *et al.*, 2007; Webster *et al.*, 2001 & Hoffman *et al.*, 1998) and it has been shown that increased genetic diversity in *Biomphalaria* snails reduces the overall *S. mansoni* transmission rate (Standley *et al.*, 2014). Webster *et al.* (2001) asserts that spatial and temporal heterogeneity in host metapopulation dynamics may instigate variable selection pressure for co-evolutionary interactions between snail resistance and schistosome infectivity and virulence. It is often predicted that heterozygosity (genetic variability) of snails enhances their fitness in the face of parasitism (Sandland *et al.*, 2007). *Biomphalaria* snails that are less heterozygous are expected to be less resistant to schistosome infection (Standley *et al.* 2014).

1.8 SNAIL- PARASITE GENOMICS

It has been established that genetics and genomics of *Biomphalaria* snails has a role to play in their interaction with *S. mansoni*. It has been suggested that there exists *Biomphalaria* strains that are naturally resistant to *S. mansoni* infections and those that are naturally susceptible to the infections, affirming that resistance is hereditary and that the genetics of the snails has a role to play in their susceptibility and resistance to the infections (Bu *et al.*, 2022; Knight *et al.*, 1999; Tennesen *et al.*, 2020; Tennesen *et al.*, 2015a; Tennesen *et al.*, 2015b).

Most genomic and genetic studies on *S. mansoni* – *Biomphalaria* interactions have focussed on *B. glabrata*. Schistosome resistant loci that have so far been identified in *B. glabrata* include OPM-04, OPZ-11, GRC, RADres, *sod1* and *PTC2* (Knight *et al.* 1999; Tennesen *et al.* 2015a; Tennesen *et al.* 2015b; Tennesen *et al.* 2020). Recently, genomic information of a second *Biomphalaria* species, *B. straminea* has been elucidated (Nong *et al.*, 2022).

Understanding the genomics of *Biomphalaria* snails can help in coming up with better control measures as far as fighting schistosomiasis is concerned. It is possible to come up with novel drugs that target specific regions of the genome with an intention of reducing the number of snails that harbour *S. mansoni* parasites. It is also possible to manipulate the interaction between the parasite and the snail, courtesy of a better understanding of the snail's genome (Tennesen *et al.*, 2020).

A study by Tennessen *et al.* (2020) on *B. glabrata* identified a genomic region called PTC2 which was established to exhibit the largest known correlation with *S. mansoni* infection and which possibly mediates host – parasite interaction at the surface level, offering potential targets for interrupting the transmission of schistosomiasis. The PTC2 region consists of genes which encode for proteins that recognize *S. mansoni* parasites when they come in contact with the snails. Manipulation of the genes in that region can therefore make it impossible for the snails to recognise the parasite and therefore, prevent infection and transmission from taking place.

Two new *B. glabrata* lines have been generated that are homozygous, with one being fully susceptible (IMline) and the other one (iBS90) being fully susceptible to *S. mansoni* infections. These *B. glabrata* lines were generated courtesy of consecutive generations of self-fertilisation. Phenotypically, snails of the IM line are albinos while on the other hand, snails of IBS90 have pigmented bodies (Bu *et al.*, 2022). These two lines can be exploited for a better understanding of the genetic mechanism underlying compatibility between *Biomphalaria* snails and *S. mansoni*.

1.9 IMMUNE REACTION OF *BIOMPHALARIA* SNAILS TO SCHISTOSOMES

Immunological reactions normally occur when *Biomphalaria* snails are infected by Schistosomes. A group of proteins called Fibrinogen Related Proteins (FREPs) that are found in the haemolymph of *B. glabrata* snails have been reported to upregulate following exposure to Schistosomes. It has also been observed that the FREPs do bind to the trematode's larval surfaces, indicative of an act of internal defence (Zhang & Loker, 2004). It has been affirmed that FREPs function in innate immunity in invertebrates (Adema *et al.*, 1997). In a study of the haemolymph of *B. glabrata* by Adema *et al.* (1997), there was a release of a precipitate following a digenetic trematode infection, whereas for uninfected snails, there was no release of a precipitate. The precipitate is indicative of the snail's immune system reaction to digenetic trematode infection by increasing synthesis of humoral defence proteins.

A study was conducted by Hertel *et al.* (2005) on two different strains of *B. glabrata* to examine the reaction of the FREPs of the two strains once the snails are exposed to *S. mansoni* infection. M line strain of *B. glabrata* is known to be susceptible to *S. mansoni* infection whereas BS-90 strain is known to be resistant to schistosome infection. There wasn't an increase in expression of FREPs in the M line after exposure to *S. mansoni* whereas in BS-90 snails, there was an increase in the expression of FREPs after exposure to *S. mansoni* (up to 57 fold increase of FREPs expression), indicative that the BS-90 strain recognizes and vigorously responds to the parasite. A transcriptome study of *B. pfeifferi* snails collected from Kenya found out that FREP2 (one of the FREP molecules) was consistently upregulated after *S. mansoni* infection whereas FREPs 5,7 and 14 were upregulated during the early infection stage and during the cercarial shedding stage (Buddenborg *et al.*, 2017). After recognition of *S. mansoni* parasite by the resistant snail strain, sporocysts are often encapsulated and killed. The killing process involves the FREPs binding to the sporocysts in a carbohydrate- inhibitable manner and acting as opsonins to facilitate phagocytosis of particles by haemocytes (Lu *et al.*, 2020).

S. mansoni possess antigens known as *S. mansoni* polymorphic mucins (SmPoMucs) which are strongly believed to interact with *Biomphalaria* snails' immune receptors (Fibrinogen related proteins –FREPs) and the nature of the interaction that occurs defines the compatible/incompatible status of *Biomphalaria* / *S.mansoni* combination. This aspect of host/parasite interactions is generally described as compatibility polymorphism (Portet *et al.*, 2017). *S. mansoni* polymorphic mucins exhibit a high level of diversification as a way of evading recognition by the invertebrate host's immune receptors. The more diversified *S. mansoni* antigens are, the higher chances of a successful parasitic infection (Gourbal *et al.*, 2015). A study of SmPoMucs at the genomic level shows that SmPoMuc is coded by a multigene family whose members frequently recombine giving rise to a high level of polymorphism based on the low number of genes (*et al.*, 2008). The high level of polymorphism exhibited by SmPoMuc based on a few numbers of genes contrasts with the polymorphism exhibited by other protozoan parasites such as *Trypanosoma cruzi* and *Plasmodium falciparum* which arise from a large set of genes (Roger *et al.*, 2008).

1.10 STUDY SITES

The study involved fieldwork in East Africa (Kenya, Uganda, and Tanzania) and lab work at the University of Nottingham, United Kingdom.

1.11 OPPORTUNITIES FOR RESEARCH ON *BIOMPHALARIA* AND *S. MANSONI*

It is worthwhile to study the geographical distribution of *Biomphalaria* snails in East Africa's River systems. Most studies on *Biomphalaria* snails in East Africa have focussed on Lake Victoria and Lake Albert and neglected the river systems. Additionally, most studies on the distribution of *Biomphalaria* snails in East Africa have been isolated projects limited in scope to single administrative areas such as a district, county, or province. It is also important to increase our understanding on the role that the population genetic structure of *Biomphalaria* snails has on the success of schistosome transmission. Furthermore, the role that genetic variation in *S. mansoni* populations has on development of drug resistance to praziquantel should be investigated. There is also a need to better understood the use of molecular tools for the detection of schistosome infections in snail intermediate hosts as a better surveillance approach, when compared to traditional surveillance methods that are parasitological in nature and detect schistosome eggs in human faecal and urine samples.

1.11.1 Geographical information systems (GIS) and spatial statistical tools for mapping intermediate host distributions

Geographical Information Systems have in recent years gained popularity in epidemiology and public health studies (Robinson, 2000). Spatial statistical tools for mapping intermediate host distributions can be used to assist in making decisions on appropriate disease intervention strategies. As far as schistosomiasis is concerned, GIS and Spatial statistical tools can be used to improve our understanding of the distribution of snail intermediate hosts and help in schistosomiasis control efforts in East Africa (Simoonga *et al.*, 2009).

Species distribution modelling continues to play a major role in predicting the potential distribution of snail intermediate hosts (Yang *et al.*, 2018). The most adopted Species distribution model in ecological studies is arguably Maximum Entropy probability

distribution Model. Maximum Entropy (MaxEnt) normally utilises occurrence records of a species of interest from online databases together with bioclimatic variables from respective databases to make predictions on the potential distribution of a species, based on their habitat suitability (Phillips *et al.*, 2006),

1.11.2 'DNA barcoding': A method for rapid species identification

DNA Barcoding is a method used to identify species by use of short DNA sequences that are shared between all or most organisms. DNA barcoding as a method for identification of organisms was initiated in 2004. For a gene region to be practically used as a DNA barcode it must meet the following criteria: the gene region should exhibit high levels of genetic divergence and genetic variability, the region should have flanking sites that are conserved and that can be used to develop universal primers and lastly, the gene region should have a short sequence length that can easily be extracted and amplified (Kress & Erickson, 2008).

One of the genes that has been identified as suitable for the identification of organisms is Cytochrome Oxidase One (CO1) gene of the mitochondrial DNA. The mitochondrial gene is found in all organisms, and it has been shown to express a high level of variation that can be used to distinguish closely related species. The 'Folmer' fragment of CO1 (which has 648 base pairs) as well as the ASMIT fragment can be used to identify species because they both exhibit high genetic variability (Herbert *et al.*, 2003). It was predicted by Herbert *et al.* (2003) that CO1 sequences would be very vital in subsequent years in the identification of organisms and revelation of genetic differences that exist between closely related species.

In addition to the use of a mitochondrial marker, use of a nuclear marker as a second marker would be important as far as comprehensive DNA barcoding is concerned. Nuclear DNA genes and regions that have been used in the past and could potentially be used in this study are the 16S rRNA gene and the Internal Transcribed Spacer (ITS) region. The 16S rRNA gene is a highly conserved gene among different species. The 16SrRNA gene and ITS region have been used in the past in population genetics and phylogenetic studies on *Biomphalaria* snails (Standley *et al.*, 2011).

One of the limitations of DNA barcoding is applying the method to taxa that exhibits extreme divergences of mitochondrial DNA sequences within species. In addition, errors in taxonomic assignment are likely to occur in cases where there is a low degree of mitochondrial variation and a small GenBank sample size (less than 25 entries per species in a reference database) (Davison *et al.*, 2009). Misidentifications in barcoding can also be caused by a wide overlap in genetic variability within and between species (Meier *et al.*, 2006). It is advised to use DNA barcoding only when conventional taxonomy and sample DNA sequences have truly identified the real identity of a given species (Davison *et al.*, 2009).

The Internal Transcribed Spacer had for a long time been viewed as a possible barcoding region to replace mitochondrial genes as an alternative in molecular systematics. However, it has been demonstrated that in the Internal Transcribed Spacer 2 (ITS-2), the lower limits of the interspecific DNA divergence ranges sometimes overlap with intraspecific range and therefore, the use of ITS-2 as a barcode in Metazoa could be problematic (Oliverio *et al.*, 2009). A study by Breugelmans *et al.* (2013) failed to differentiate two species of the

terrestrial slugs *Arion* by use of the Internal Transcribed Spacer 1 (ITS-1) as a barcode thus implying that ITS-2 may equally not be highly reliable in barcoding of snails.

1.11.3 Molecular detection of *S. mansoni* infections in *Biomphalaria* snails

Rapid monitoring and surveillance of schistosome infections is required if schistosomiasis is to be eliminated. Development of PCR techniques that are rapid in nature have made it possible to identify gene markers with ease and at a fast pace. In spite of the development of the PCR techniques, they have not been properly applied in low resource settings of tropical and subtropical countries, where schistosomiasis is endemic. The low use of these PCR techniques could partly be because not all these localities are connected to the electric grid and molecular labs are often not in close proximity to such areas.

Nonetheless, due to the rapid nature of PCR techniques, the techniques can provide an alternative monitoring approach on schistosomiasis that is accurate and time saving.

In this study, we propose and apply a PCR technique developed by Sandoval *et al.* (2006) and a PCR technique developed by Lu *et al.* (2016) to detect *S. mansoni* infections in *Biomphalaria* snails found in East Africa's River systems.

1.11.4 Population genetic studies of *Biomphalaria* populations found in East Africa's River systems

It is important to understand the population genetic structure of different *Biomphalaria* populations. Investigating the population genetic structure of *Biomphalaria* snails in East Africa's River systems can help us understand the possible evolutionary forces that affect the genetic structuring process of the populations. Phylogenetic relationships of different *Biomphalaria* snails in the river systems can also be revealed courtesy of population genetic studies.

Biomphalaria snails reproduce both asexually and sexually. Depending on the type of reproduction that is embraced by a given *Biomphalaria* population, this will play a key role in the genetic structuring process of that particular population. Sexual reproduction plays a major role in ensuring genetic mixing and emergence of novel gene combinations in each offspring. The novel gene combinations in the offspring have the potential to help them adapt to selective pressures leading to diverse and successful populations (Rey *et al.*, 2021).

1.11.5 Population genetic studies of *S. mansoni* parasites found in *Biomphalaria* snails

Investigating the population genetics of *Schistosoma* parasites is important because this may reveal the different selection pressures that have the potential to change the evolution path of the parasites. Selection pressures that are likely to change the evolution path of *Schistosoma* parasites include habitat change, pollutants, migration, and prevention/control interventions (Rey *et al.*, 2021).

Most studies on the population genetics of *Schistosoma* parasites have involved free-living adult parasites and not prepatent larval stages in snail intermediate hosts. Very few studies have studied the population genetics of *S. mansoni* parasites harboured in

Biomphalaria snails. We saw it an opportunity to study the population genetics of *S. mansoni* parasites in *Biomphalaria* snails.

1.12 RESEARCH AIMS

The general introduction on Schistosomiasis, *Schistosoma* parasites, *S. mansoni*, prevention and control of schistosomiasis and *Biomphalaria* snails has addressed a number of areas that would be better understood if further research is conducted.

1. What is the distribution of *Biomphalaria* snails in East Africa's River systems?

Biomphalaria snails in East Africa's River systems are understudied compared to *Biomphalaria* snails found in lakes, more so Lake Victoria and Lake Albert. A survey was conducted by Brooker *et al.* (2009) to establish the geographical distribution of *S. mansoni* and *S. haematobium* in East Africa but few studies have been conducted to extensively establish the geographical distribution of *Biomphalaria* snails in East Africa's River systems.

Understanding the distribution of *Biomphalaria* snails in East Africa's River systems would provide cues on the prevalence of human intestinal schistosomiasis. Areas where *Biomphalaria* snails will be found would be important in delineating potential hotspots as far as transmission of human intestinal schistosomiasis is concerned. Understanding the distribution of *Biomphalaria* snails in the river systems would also influence decision making processes in coming up with effective and integrated strategies to combat the transmission of human intestinal schistosomiasis. Malacological surveys of *Biomphalaria* snails would also provide more information about the environmental factors that are conducive for the distribution of the snails. Distribution of *Biomphalaria* snails is not only affected by environmental factors but also biological factors such as presence of competitor snails; the study will also reveal competitor snails that affect the distribution of *Biomphalaria* snails in East Africa's River systems. Some of the competitor snails that have been revealed to be a threat to the existence of *Biomphalaria* snails include *Melanoides tuberculata*, *Thiara granifera*, *Ampullaria glauca* and *Marisa cornuarietis* (Toledo & Fried, 2011).

2. What is the population genetic structure of *Biomphalaria* snails in East Africa's River systems?

There is a need to understand the population genetic structure of *Biomphalaria* snails found in East Africa's rivers systems. It is important to reveal the genetic distances between the different *Biomphalaria* populations. This will help us predict the possible role of several selection pressures on the genetic structuring process of the *Biomphalaria* populations. Population genetic studies will also help us understand the potential role of mating, migration, geographical isolation, gene flow and parasitism on the genetic structure of the *Biomphalaria* populations.

Given that several studies have shown that the population genetic structure of *Biomphalaria* snails has a role to play in the success of schistosome infections; the knowledge obtained here in can help in developing proper schistosomiasis control and prevention strategies.

3. What is the prevalence of *S. mansoni* infections in *Biomphalaria* snails found in East Africa's River systems?

Surveillance and diagnosis of schistosome infections by detecting schistosomiasis in human beings can be a lengthy, tiresome, and complex process. Furthermore, there are many human beings in endemic areas who blatantly refuse to engage in surveillance and diagnostic studies because of different reasons such as time constraint, negative myths and beliefs about the disease and lack of trust on the entire process.

The detection of schistosome infection in snail intermediate hosts found in endemic areas can help overcome challenges faced by the traditional surveillance and diagnostic approaches. Snails do not have negative beliefs on schistosomiasis, they do not have time constraints and they 'can't fail to cooperate' in a study. The tentative prevalence of human intestinal schistosomiasis in a given geographical area can be indirectly established by detecting schistosomes in snail intermediate hosts.

4. What is the genetic structure of *S. mansoni* populations in schistosome infected *Biomphalaria* populations found in East Africa's River systems?

Few studies have explored the population genetic structure of prepatent and sub-patent *S. mansoni* populations. Exploring the population genetic structure of the *S. mansoni* populations through this approach can be a time saving way of understanding the population genetic structure of different *S. mansoni* populations.

It has been revealed that the population genetic structure of *Schistosoma* parasites has a role to play on their potential resistance to praziquantel and other drugs used for treatment of schistosomiasis in human beings. Therefore, the population genetic structure of *S. mansoni* parasites in a given geographical area can help us forecast the success rate of mass drug administration programmes in that particular locality. It has also been revealed that mass drug administration programmes can serve as a selective pressure that can contribute to possible evolutionary potential of *Schistosoma* parasites.

CHAPTER TWO: METHODS

2.1 Species distribution modelling and fieldwork

2.1.1 Species distribution modelling

Species distribution modelling was used to predict where to sample *Biomphalaria* snails. Occurrence records of sites where *Biomphalaria* snails have previously been found was obtained from GBIF-Global Biodiversity Information Facility (GBIF, last accessed in December 2017). A total of 100 occurrence records were retrieved from GBIF (Supplementary Figure 1 and Supplementary Table 1). The occurrence records were for the entire African continent and not only East Africa.

Bioclimatic variables were obtained from [WorldClim.org](https://worldclim.org) and soil type and land cover variables obtained from respective databases. We obtained 19 Bioclimatic variables from <https://worldclim.org/> at a spatial resolution of 2.5 arc-min. The Bioclimatic variables represent seasonality, annual trends, and extreme climatic conditions. The Bioclimatic variables have been widely applied in making predictions on the potential distribution of several species (Guo *et al.*, 2013; Mainali *et al.*, 2015).

Soil type variables and landcover variables were also used in the study. Soil type variables were derived from <https://www.isric.org/explore/soil-geographic-databases> and landcover variables were derived from <http://2016africallandcover20m.esrin.esa.int/download.php>

Ecological modelling was done using MaxEnt (Phillips *et al.*, 2006), a machine learning algorithm that is used to predict the potential distribution of species, based on the principle of maximum entropy in a situation where there is incomplete information about the distribution of a given species and the environmental factors that promote its habitation. To conduct maximum entropy species distribution modelling, occurrence points-data (sites where *Biomphalaria* snails have been found previously) and environmental layers (bioclimatic factors and environmental factors that affect the distribution of species) are required.

Maxent has a better performance rate when compared to other species distribution models, in a situation where prediction has to be done yet we lack total information about the distribution of the species as well as the environmental factors that affect the distribution of the snails (Elith *et al.*, 2006). Selection of relevant predictor variables and a set of factors associated with habitat suitability is important in species distribution modelling (Warren & Seifert, 2011).

2.1.2 Fieldwork: malacological surveys

MaxEnt predictions and past knowledge on the distribution of *Biomphalaria* snails in East Africa's River systems guided where we undertook our malacological surveys. Additionally, we visited water bodies in regions that have previously been reported as having cases of intestinal schistosomiasis, on the basis that there must be snail intermediate hosts that transmit the disease in those localities.

Malacological surveys were conducted across Kenya, Uganda and Tanzania in search of *Biomphalaria* snails. Sampling was undertaken from January to March and November to December in 2018, January to March 2019 and January to March 2020. These months were

selected as more suitable for sampling snails because historically, they are months with light rains (not extreme weather conditions such as floods and drought). Various types of habitats were visited during the malacological surveys including rivers, irrigation schemes, dams, streams and springs with surveys undertaken at 72 sites in Kenya, 23 sites in Uganda and 77 sites in Tanzania. A total of 172 sites were visited across East Africa's River system, in search of *Biomphalaria* snails (Supplementary Table). We used public transport (buses, taxis and motorbikes) to visit the sites. The surveys involved searching water bodies for approximately 20 minutes, covering approximately a distance of 50 metres and collecting all the *Biomphalaria* snails that were found, following procedures adopted by Standley *et al.*, (2014). Collection of the snails was conducted using a metallic scoop. Snails that were found were collected using a handheld scoop (Figure 18). Various types of habitats were visited during the malacological surveys including rivers, irrigation schemes, dams, streams and springs (Figure 19). We did not have a microscope in the field and therefore could not conduct cercarial shedding studies.

Geographical coordinates and altitude value for each sampled site was recorded using a handheld Garmin eTrex 10 GPS (Kansas City, USA). The snails were then sorted by turning them upside down on the wire mesh of the collection scoop to check their shell characters. Identification keys developed by Brown, (1994); Kristensen (1987) & Mandahl, (1957) were used to identify *Biomphalaria* snails based on their morphological characters including the general shape of the shell, the shape of the whorls, the number of coils and the shape of the aperture. Genital morphology was not used in the identification of the snails as it does not constitute one of the parameters highlighted in the identification keys. Juvenile snails were not collected.

Ecological and physicochemical parameters associated with snail abundance were also recorded. These parameters included vegetation type, water temperature and pH. Water temperature and pH were recorded using a portable water metre (Hanna Instruments, Møllevænget, Sweden). Water depth was also recorded using a 1 metre ruler. Furthermore, water velocity was recorded using a flowmeter (a current meter which turns with the flow of a stream or a river). Additionally, we noted and recorded the soil type for each site. Supplementary Table 2 lists all the sites that were visited for malacological surveys, including their respective geographical coordinates, altitude, ecological information, and physicochemical information.

Snails were transferred to a collection jar then washed and placed in Falcon tubes while in the field. To preserve the snails, absolute ethanol was added to the lab bottles containing the snails to a volume double that occupied by the snails. Ethanol in the lab bottles was changed two to three times, to ensure that ethanol penetrated into the snails' tissues. After completion of fieldwork, the lab bottles containing the snails were stored in a - 20°C freezer.



Figure 18: Snail sampling Images.



Figure 19: Different types of habitats from which the snails were sampled. A. River (Ibulwa River- Tanzania), B. Dam (Kangonde dam -Kenya, C. Stream (K'Otieno stream -Kenya), D. Irrigation scheme (Mabogini Irrigation scheme – Tanzania), E. Spring (Opiyai Angorom- Uganda).

2.1.3 Maps creation and testing of physicochemical and ecological parameters in relation to snail abundance

Maps showing sites that were visited and sites where *Biomphalaria* snails were found in relation to snail abundance were created by QGIS version 3.12.3 (Figure 20). The name of the site, geographical coordinates, altitude, availability of snails, snail numbers, ecological and physicochemical parameters for each site were recorded for the purpose of determining ecological and physicochemical factors associated with snail abundance, the Gaussian log function was used. In this function, *B. pfeifferi* snail abundance was used as an outcome while independent variables were the physicochemical and ecological parameters. Negative Binomial Generalised linear mixed models (GLMMs) were fitted in R Version 4.1.0 using the package 'glmmTMB' to test association between snail abundance and parameters. The comparisons were made on all 172 sites, not just the 31 sites where *Biomphalaria* snails were found.

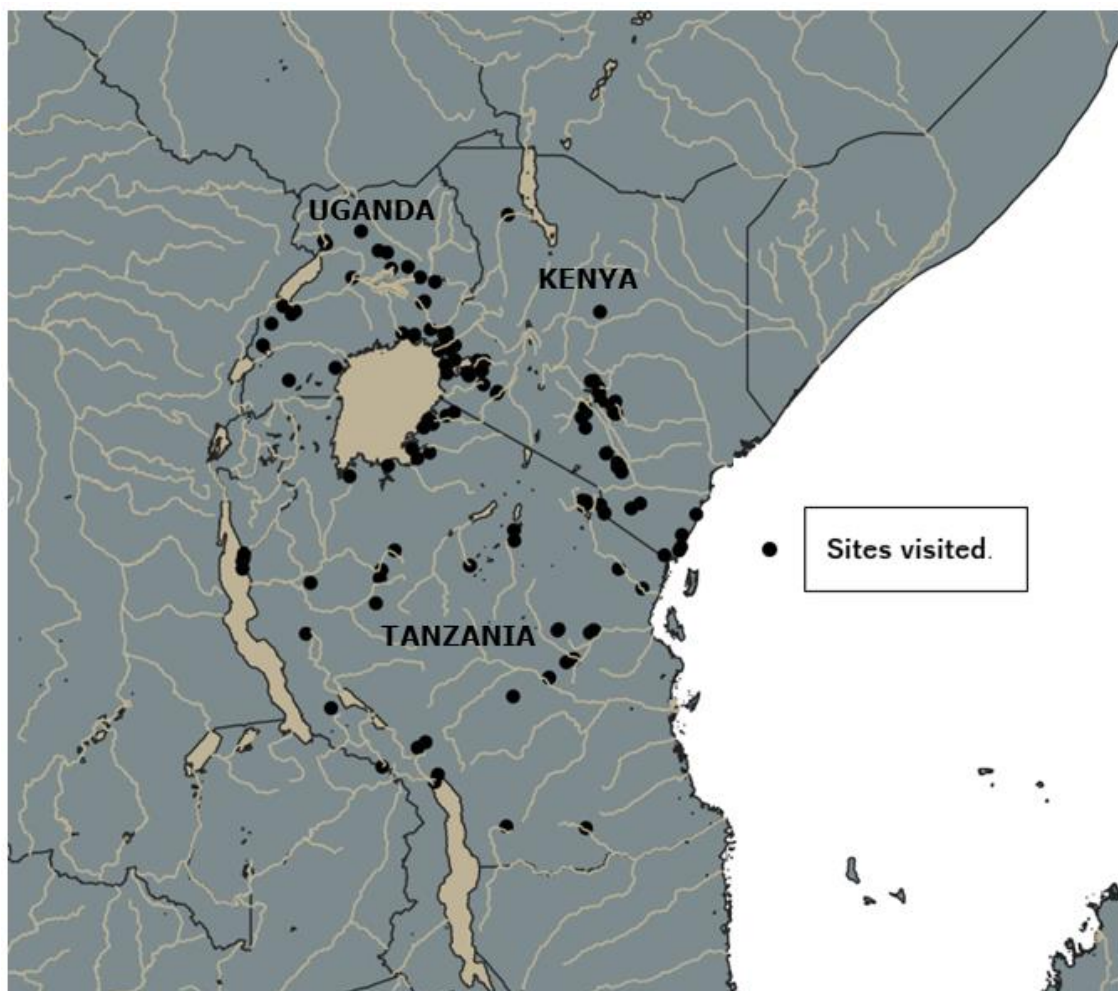


Figure 20: A map of East Africa showing sites that were visited for purposes of malacological surveys.

2.2 DNA extraction

An average of 10-12 individuals per each *Biomphalaria* population were used in the DNA extraction process. DNA was extracted from snails using the CTAB (hexadecyltrimethylammonium bromide) method (Goodacre & Wade, 2001). A slice of soft tissue from each snail was cut into pieces and placed in 1.5ml eppendorf tubes containing 500ul CTAB buffer (100 mM Tris base, 20 mM EDTA, 1.4 M NaCl, 2% CTAB). Glass beads were added to the tubes and the tissue grinded with plastic pestles. This was followed by addition of 20µl proteinase K (10g/ml concentration) and 1ul of 2-mercaptoethanol. The tubes were incubated at 55°C for 2 – 3 hours until all tissues dissolved. 500µl of chloroform-isoamyl alcohol (24:1) were then added, mixing of the tubes was done and then the tubes inverted for 5 minutes before being spun at 13000 rpm for 5 minutes. The top aqueous layer containing the DNA was collected and placed into pre-labelled 1.5ml tubes. 1ml of absolute ethanol and 4ul of sodium acetate were then added to each mixture and left at -80 overnight (about 12 hours) to precipitate. The mixtures were spun at 13000 rpm before the removal of ethanol leaving the DNA pellets. The DNA pellets were washed with 70% ethanol, and then allowed to air-dry. The DNA pellets were re-suspended in 200ul Tris buffer and stored under -20°C until use.

2.3. Selection of molecular markers

The degree of genetic diversity and genetic population differentiation of the *Biomphalaria* populations was deduced from genetic variation in a mitochondrial gene (cytochrome oxidase sub-unit one (CO1)) and the Internal transcribed spacer 2 region of the rRNA gene) (ITS-2).

Cytochrome Oxidase subunit one (CO1) gene is a fragment of mitochondrial DNA that shows high evolutionary rates of the mitochondrial genome. CO1 has been used in many population genetics studies of Class Gastropoda. It is a useful marker for phylogenetic relationships analysis at the species level. It has been established that CO1 shows a high resolution in revealing phylogenetic relationships of species that are closely related (Elejalde *et al.*, 2005). The internal transcribed spacer 2 (ITS-2) region of nuclear ribosomal DNA is used in DNA barcoding because of its ease of amplification and ability to reveal variability in closely related species (Hui *et al.*, 2010).

2.4 Amplification of molecular DNA markers

To amplify mitochondrial DNA, Polymerase Chain Reactions (PCRs) were conducted using Bioline MyTaq™ polymerase (BIO-21105) in combination with LCO and HCO primers to amplify Cytochrome c Oxidase subunit I [COI]. LCO 1490: (5' -GG TCAACAAATCATAAAGATATTGG-3') and HCO 2198: (5'-TA AACTTCAGGGTGACCAAAAATCA-3') primers are universal primers developed by Folmer *et al* (1994) for amplification of mitochondrial Cytochrome c Oxidase subunit 1 from metazoan invertebrates. PCR cycling conditions involved an initial denaturation step of 5 minutes at 95°C, followed by 40 cycles of 45 s at 94°C, 1 min at 55°C and 1 min at 73°C. The cycling ended with a 7 min extension step at 73°C. Reactions were held at 4°C. A negative control containing only reaction mixture components was used to identify potential contamination by being included with samples containing DNA in each amplification process.

To amplify Internal Transcribed Spacer 2 (ITS-2), ITS-2F, the forward (F) primer (5'-CGTCCGTCTGAGGGTCGGTTTGC-3') anchored in the 5.8 region and the reverse (R) primer ETTS1 (5'-TGCTTAAGTTCAGCGGGT-3') anchored in the 28S region were used in the study. Initial denaturation step was for 3 min at 95°C and then 32 cycles with annealing at 54°C for 1 min, extension at 72°C for 2 min, denaturation at 95°C for 45s, and a final extension step at 72°C for 5 min. A negative control (no DNA) was included in all experiments.

2.5 Visualising PCR Products and DNA sequencing

PCR products were run on a 1.5% agarose gel stained with ethidium bromide for verification of the amplifications. Verified PCR amplicons were sent to MacroGen Europe for purification and SANGER sequencing.

2.6 Phylogenetic and population genetics analysis

Phylogenetic and Population genetic analyses were executed with an intention to probe the identity, genetic diversity, population structure and genetic population differentiation of the *Biomphalaria* populations that were surveyed. Sequence trace data was checked and edited in Finch TV (Geospiza, 2021) and CO1, ITS-2 and ND5 sequences aligned using Seaview version 5.0 (Gouy *et al.*, 2021). DnaSP6 (Rozas *et al.*, 2017) was used to generate haplotype data from the aligned sequences for each *Biomphalaria* species found; this includes haplotype distribution, the number of haplotypes and haplotype diversity.

Phylogenetic relationships among the species of *Biomphalaria* snails and *Schistosoma* parasites were deduced by PhyML version 2.4.4 using the Maximum Likelihood method, using the GTR+G model, and with bootstrap values based on 1000 replicates (Guindon & Gascuel, 2003). CO1 and ITS-2 sequences of African *Biomphalaria* snails submitted in Genbank were introduced in the study to establish the position of the 'new' sequences in respective phylogenetic trees. For both the CO1 and ITS-2 trees, the tree was rooted on a *B. glabrata* sequence (Accession numbers-MK395976 CO1 and AY030374 ITS-2). The basis of rooting both the trees on *B. glabrata* sequences is because it has been established that *B. glabrata* is the ancestor of the African *Biomphalaria* snails (DeJong *et al.*, 2003). For the phylogenetic tree of *Schistosoma* parasites, ND5 sequences from our fieldwork study and those from GenBank belonging to different *Schistosoma* species were used. The *Schistosoma* tree was rooted on *Taenia solium* (Accession number- MW718881).

A median joining network was generated for *Biomphalaria* species in PopArt version 1.7 to reveal the relationship that exists between the haplotypes (Leigh *et al.*, 2015). Measures of population differentiation were calculated from CO1 sequence data, ITS-2 and ND5 sequence data by use of Analysis of Molecular Variance (AMOVA) and FST values. Genetic diversity within the populations was determined by examining haplotype diversity (h) and nucleotide diversity (π) (Arlequin version 3.5, Excoffier and Lischer 2010). AMOVA (Analysis of Molecular Variance) was calculated using Arlequin version 3.5 so were FST values. AMOVA was calculated for the *B. pfeifferi* group and the Nilotic species complex group separately by first comparing all the populations for each species group across the three countries and then comparing the populations grouped as members of a group of populations in line with the country (comparing genetic variation in populations grouped based on their country of origin). Pairwise comparisons across CO1 and ITS-2 sequence data respectively, were also computed in Arlequin version 3.5.

2.7 Schistosome infection detection

S. mansoni specific primers designed by Lu *et al.* (2016) amplifying the ND5 gene (5'-ATT AGA GGC AAT GCG TGC TC-3') and reverse (5'-ATT GAA CCA ACC CCA AAT CA-3') were used to detect schistosome infection in *Biomphalaria* snails. *S. mansoni* primers designed by Sandoval *et al.* (2006) amplifying the 28S rDNA region (5'-GAGATCAAGTGTGACAGTTTTGC -3') and reverse (5'-ACAGTGCGCGCGTCGTAAGC- 3') were equally used for schistosome infection detection in *Biomphalaria* snails.

PCR reactions were carried out in a reaction buffer, containing 2.5mM MgCl₂, 0.2mM each dNTP, 0.2μM of each primer, 1 unit of Taq polymerase (Bioline) and 1 μl of template DNA from the snail extract. The PCR cycling conditions were as follows: 35 cycles, each cycle consisting of 94 °C for 20 sec, 65 °C for 20 sec and 72 °C for 30 sec. Two positive controls containing pure *S. mansoni* DNA were used in each experiment. Two negative controls (Master mix with no DNA and Master mix with a *S. mansoni* negative snail extract) were also included in the experiments. A DNA ladder of 100 base pairs was also used in each experiment. PCR products were run on a 1.5% agarose gel (stained with ethidium bromide) for 1 hour at 100V with gels visualised using a UV transilluminator to confirm amplified products and check the position of amplified DNA with that of *S. mansoni* DNA in relation to the DNA ladder.

2.8 Data analysis for infection detection study

Data analysis was conducted using IBM SPSS Statistics. A comparison was made relating *S. mansoni* infected *Biomphalaria* populations to the countries from where they were found. The proportion of sites from where *S. mansoni* infected *Biomphalaria* populations were found in relation to the total sites visited was also calculated, at per country level. We also analysed the infection rates of the *Biomphalaria* species that were found in the River systems.

2.9 Genetic analysis of *S. mansoni* ND5 Mitochondrial gene

Sequence trace data was checked and edited in Finch TV (Geospiza, 2021). Sequences were then aligned using SeaView version 5.0 (Gouy *et al.*, 2021). DnaSP6 (Rozas *et al.*, 2017) was used to generate haplotype data from the aligned sequences; this includes haplotype distribution, the number of haplotypes and haplotype diversity. A Minimum Spanning network was then generated for each data set in PopArt version 1.7 to reveal the relationship that exists between the several haplotypes (Leigh *et al.*, 2015). Genetic diversity and measures of population differentiation were then determined by use of Analysis of Molecular Variance (AMOVA) tests and F_{ST} values using Arlequin version 3.5 (Excoffier and Lischer 2010).

CHAPTER THREE: POPULATION GENETICS OF *BIOMPHALARIA* SNAILS FOUND IN EAST AFRICA'S RIVER SYSTEMS

ABSTRACT

Investigating the population genetics of *Biomphalaria* snails in East Africa's River systems is important because the knowledge obtained from such kind of studies can help develop more appropriate intervention strategies in the fight against human schistosomiasis. The population genetic structure of *Biomphalaria* snails in East Africa's River systems remain poorly described. Here, we deduce the population genetic structure of *Biomphalaria* populations by use of a mitochondrial marker (Cytochrome Oxidase 1 gene) and a nuclear marker (ITS-2 region of nuclear ribosomal DNA). The sequence data was analysed to determine inter and intra population genetic diversity and structure of the snail populations. There was a clear geographical clustering of haplotypes with the identification of 18 *B. pfeifferi* CO1 haplotypes and 20 CO1 haplotypes for the 'Nilotic Species Complex' individuals. As for ITS-2 sequence data, there was identification of 27 *B. pfeifferi* ITS-2 haplotypes and 23 haplotypes for the 'Nilotic Species Complex' individuals. Analysis of Molecular Variance (AMOVA) revealed low genetic variation within *B. pfeifferi* populations (between 4.83% and 5.86%) and high genetic differentiation among the populations (between 94.14% and 95.17%). As for the 'Nilotic Species Complex' individuals, the genetic diversity within populations was moderate (between 28.78% and 72.17%) so was the genetic differentiation among populations (between 27.83% and 49.74%).

3.1 INTRODUCTION

Biomphalaria is a genus of freshwater, air breathing snails that belong to the Family Planorbidae. *Biomphalaria* snails are primarily found in the tropics and sub tropics: South America, Central America (Caribbean), Asia and Africa. *Biomphalaria* snails found in Africa include *Biomphalaria pfeifferi*, *Biomphalaria camerunensis*, *Biomphalaria alexandrina*, *Biomphalaria sudanica*, *Biomphalaria choanomphala*, *Biomphalaria stanleyi*, *Biomphalaria angulosa* and *Biomphalaria smithi* (Brown, 1994). Phylogenetic studies indicate that the African *Biomphalaria* species are derived from the South American species, *Biomphalaria glabrata* (Campbell *et al.*, 2000).

Biomphalaria snails are medically important serving as intermediate hosts of *Schistosoma mansoni* (Gryseels *et al.*, 2012). *S. mansoni* is the leading causative agent of human schistosomiasis, a neglected tropical disease with estimates of 250 million people infected and 1 billion people at risk of infection (WHO, 2021). It has been documented that there is a high level of variation in compatibility between *S. mansoni* and the different *Biomphalaria* species (Standley *et al.*, 2014). The difference in compatibility between *S. mansoni* and the different *Biomphalaria* snails is driven by biotic and abiotic factors that create localised transmission dynamics of *S. mansoni* infections (Mutsaka *et al.*, 2020).

Biomphalaria pfeifferi is arguably the most important snail intermediate host of *S. mansoni* in Africa because of its widespread distribution on the continent and its relatively high susceptibility to schistosome infections (Buddenborg *et al.*, 2019). In East Africa, other than *B. pfeifferi*, other *Biomphalaria* snails that have been reported to be responsible for the transmission of *S. mansoni* include *Biomphalaria sudanica*, *Biomphalaria choanomphala*, *Biomphalaria smithi*, *Biomphalaria stanleyi* and *Biomphalaria angulosa* (Rowel *et al.*, 2015).

Prevention and control efforts in the fight against schistosomiasis, target either *Schistosoma* parasites or snail intermediate hosts or both. Schistosomiasis control efforts that target snail intermediate hosts, albeit unpopular involve use of molluscicides to kill the snails consequentially interfering with the transmission of *Schistosoma* parasites to mammalian hosts (Gryseels, 2012).

The population genetic structure of snail intermediate hosts has a role to play in the success of parasitic infections. Reduced genetic diversity within *Biomphalaria* populations has been shown to increase *S. mansoni* transmission rates whereas increased genetic diversity has been shown to reduce schistosome transmission rates (Standley *et al.*, 2014; Sandland *et al.*, 2009; Wethington *et al.*, 2007; Hoffman *et al.*, 1998).

Most studies (including population genetic studies) on *Biomphalaria* snails in East Africa (Kenya, Uganda and Tanzania) have concentrated on Lake Victoria and Lake Albert (Standley *et al.*, 2011; Standley *et al.*, 2014; Rowel *et al.*, 2015) and neglected river systems. There is a need to elucidate the population genetic structure of other *Biomphalaria* snails found in East Africa other than those found in the two lakes. The two lakes constitute 4.13% (73,000 km²) of the entire geographical area of East Africa (1,766,491 km²), leaving *Biomphalaria* snails occupying 95.87% poorly studied and understood. Understanding the population genetic structure of other *Biomphalaria* snails outside of Lake Victoria and Lake Albert will help understand transmission dynamics of human intestinal schistosomiasis in

other parts of East Africa and assist in developing better control and prevention strategies in the fight against the disease. In this study, we investigate genetic diversity and population genetic structure of *Biomphalaria* populations found in East Africa's River systems.

3.2 RESULTS

3.2.1 Phylogenetic trees

Phylogenetic trees were created in PhyML software version 2.4.4, using the GTR+G model, and with bootstrap values based on 1000 replicates. The phylogenetic tree of all *Biomphalaria* CO1 data from Genbank and sequence data from our fieldwork clearly separates 'New World' *Biomphalaria* species from 'Old World' *Biomphalaria* species (Figure 21). Phylogenetic trees built using African *Biomphalaria* GenBank data and our fieldwork CO1 and ITS-2 data are shown in Figures 22,23, 24 and 25. *B. pfeifferi* forms a monophyletic group in both trees supported in 98% and 92% bootstraps for CO1 and ITS-2 respectively that falls separately to the other African *Biomphalaria* species. *Biomphalaria camerunensis* takes a basal position in the trees, suggesting that it is the oldest member of African *Biomphalaria* snails (Figure 22 and Figure 23). The CO1 tree separates into two main groups, a *B. pfeifferi* group supported in 97% of bootstrap replicates and a 'Nilotic species complex' group supported in 72%. The 'Nilotic species complex' group members are *B. sudanica*, *B. choanomphala*, *B. smithi*, *B. stanleyi*, *B. angulosa* and *B. alexandrina*. In the ITS-2 tree, the division between *B. pfeifferi* and the 'Nilotic species complex' is not well resolved. *B. choanomphala* and *B. sudanica* are completely intermingled in both trees (clustering with *B. alexandrina*, *B. smithi*, *B. stanleyi* and *B. angulosa*).

In the *B. pfeifferi* group subtree (Figure 24), our sequences from Ntoroko (Uganda) take a closer position to GenBank sequences from Sudan and Zimbabwe. Our sequences from Mwamgongo (Tanzania), Utwigu (Tanzania) and Bwawani (Tanzania) take close positions to each other in the *B. pfeifferi* group subtree. Similarly, our sequences from Western Kenya (Ambururu, Asawo, Rakite, Asawo, Kodumo, Kodongo, Cheptuyet and Onsando) take close positions to each other in the *B. pfeifferi* group subtree. Our sequences from Eastern Province Kenya (Musilili, Kakukutuine, Kalundu, Ivuta, Matingani, Tulimuimbu Itua and Kwase take close positions to each other on the *B. pfeifferi* group subtree. Our sequences from Central Kenya (Mukou and Kiangangi) cluster with our sequences from Eastern Province, Kenya.

Among our fieldwork 'Nilotic Species Complex' individuals, 3 groups can be deduced. The first group is the '*B. choanomphala* type' group, the second group is the '*B. sudanica* type' group and the third group is the '*B. stanleyi* type' group (Figure 25).

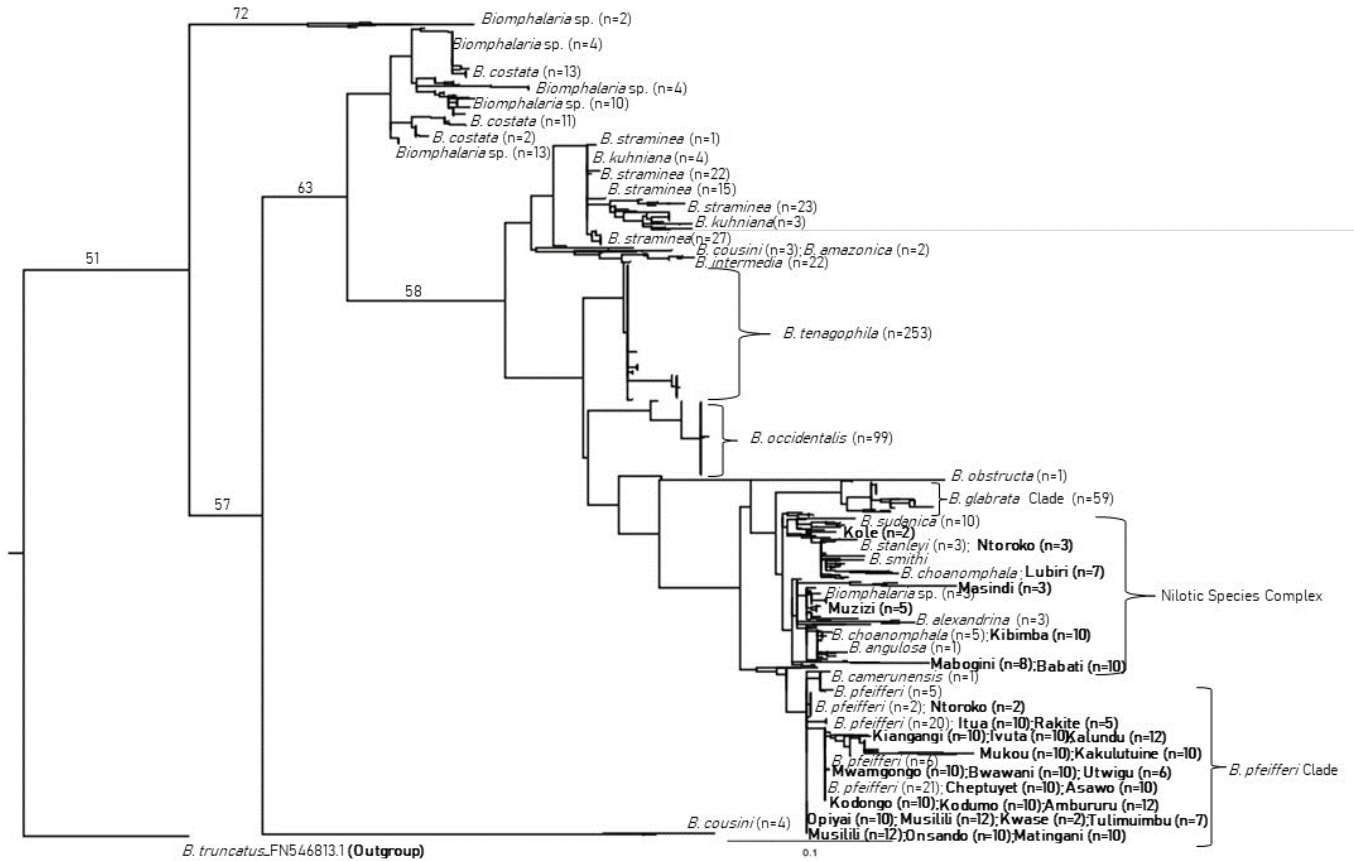


Figure 21: Maximum likelihood tree of Global *Biomphalaria* CO1 sequences. Sequences of snails collected from our fieldwork are written in bold whereas GenBank sequences are not highlighted.



Figure 22: Maximum likelihood tree of African *Biomphalaria* C01 sequences. Sequences of snails collected from our fieldwork are written in bold, with country of collection in brackets and an asterisk at the end whereas genbank sequences are not highlighted.

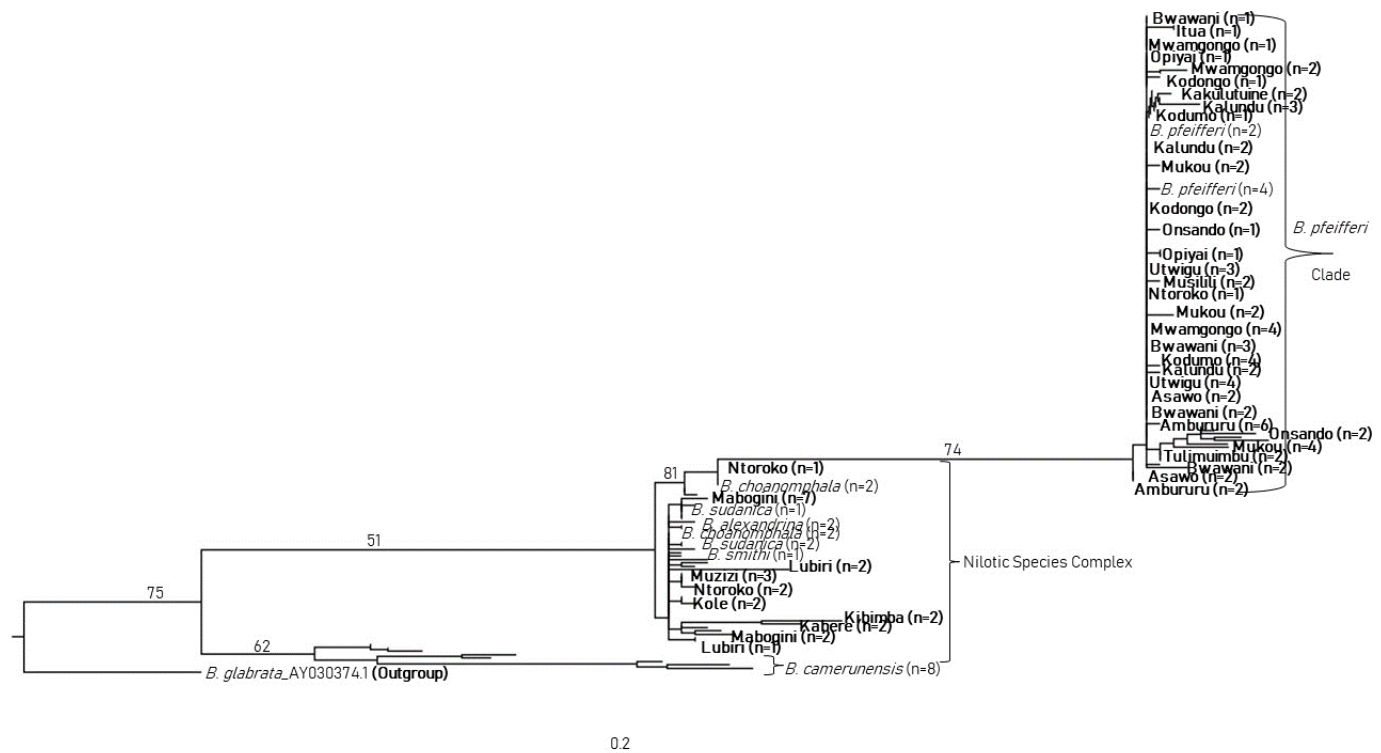


Figure 23: Maximum likelihood tree of *Biomphalaria* ITS-2 sequences. GenBank sequences have their accession numbers included in their names.

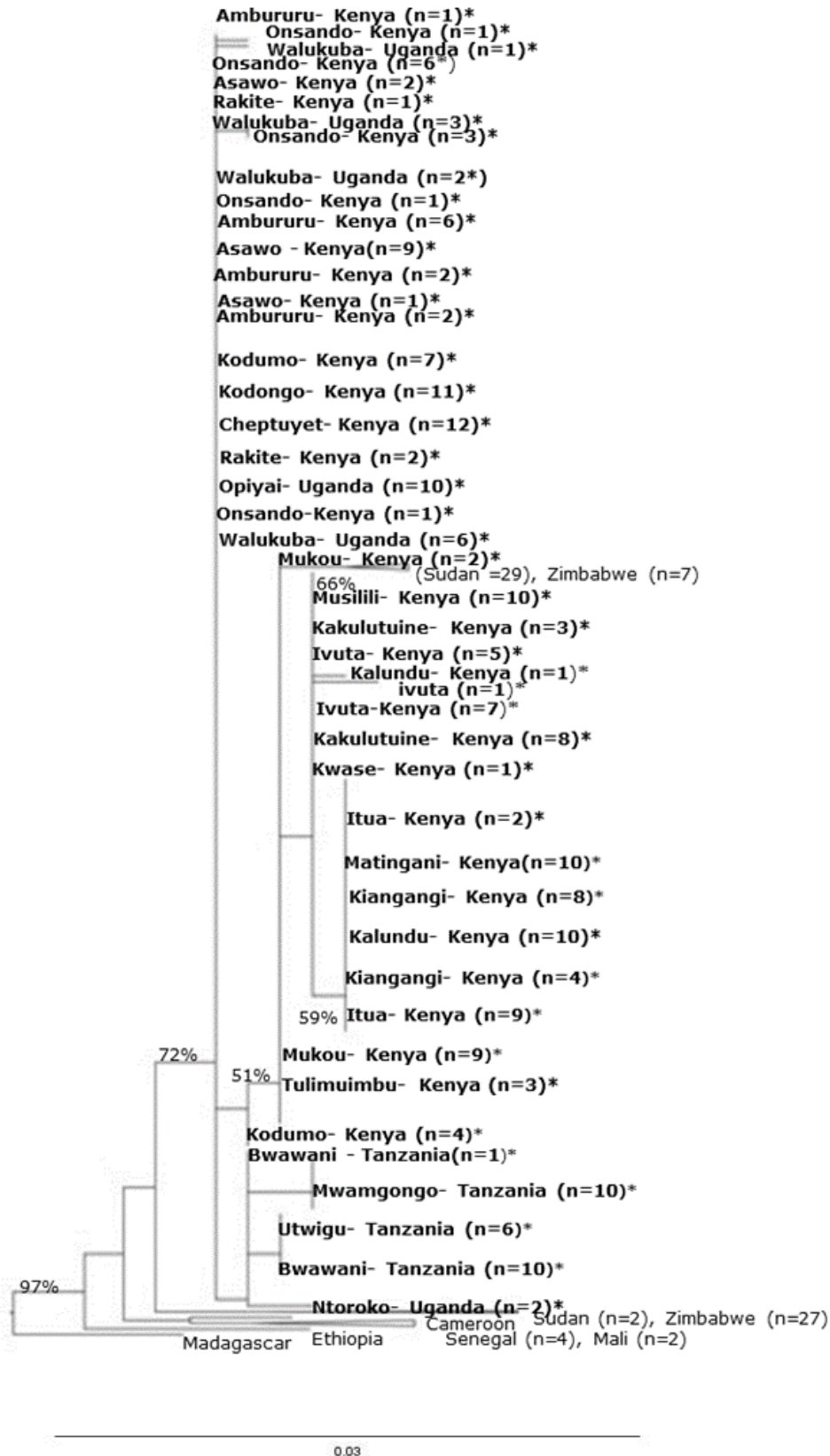


Figure 24: Maximum likelihood subtree of African *B. Pfeifferi* CO1 sequences. Bootstrap values less than 50% not shown. Genbank sequences are named after their country of origin whereas sequences of snails collected from our malacological surveys are highlighted with an *(asterisk).

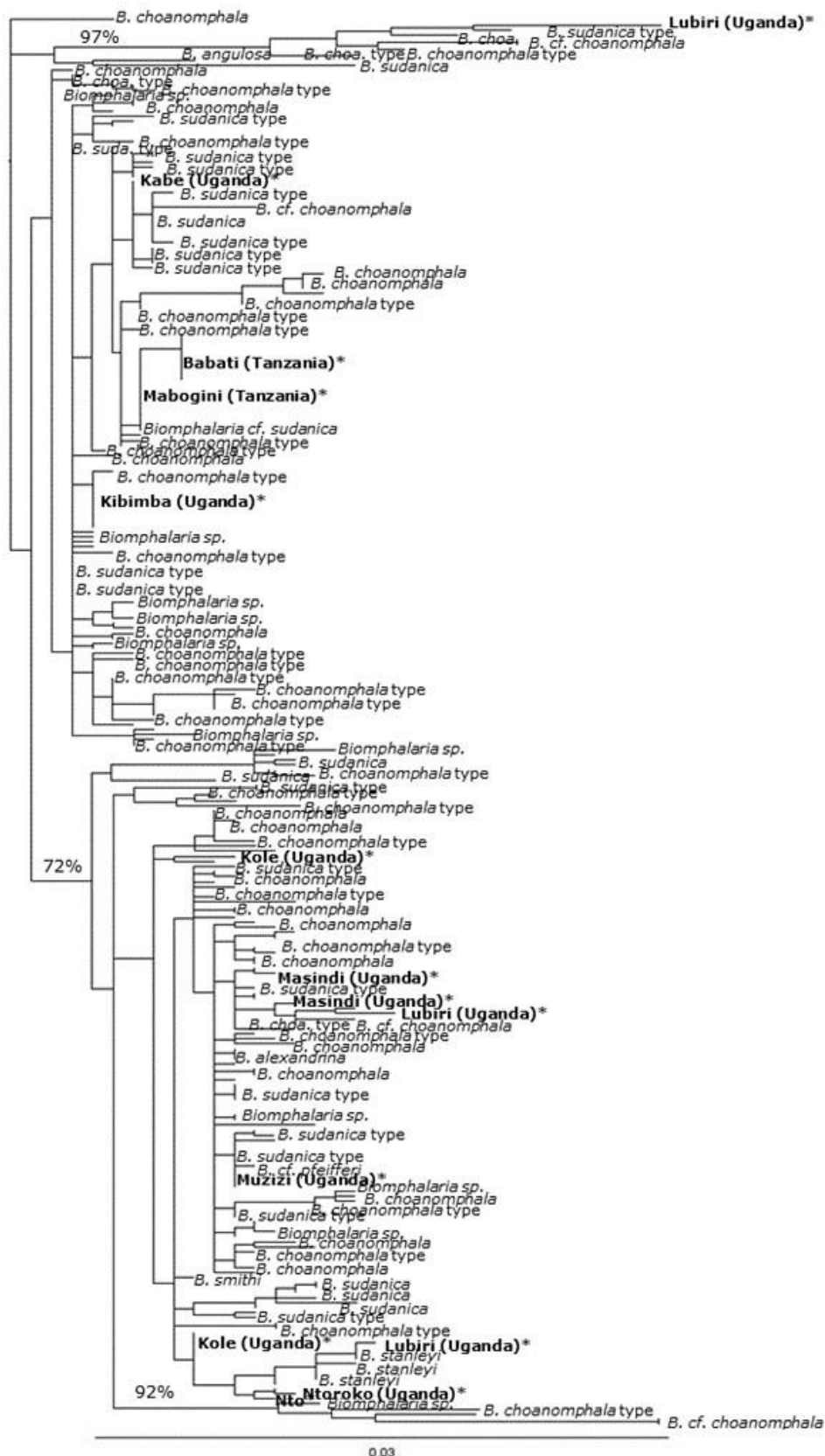


Figure 25: Maximum likelihood subtree of the 'Nilotic species complex' CO1 sequences. Bootstrap values less than 50% not shown. Sequences from our malacological surveys sites written in bold with an asterisk at the end.

3.2.2 C01 haplotypes and networks

DnaSP6 software was used to generate haplotype data information. Significant divergent haplotypes were not found within the same population, suggesting very low to moderate incidents of gene flow. A total of 17 *B. pfeifferi* C01 haplotypes with a haplotype diversity of 0.7978 and nucleotide diversity of 0.00441 were identified. Haplotypes differed based on one or two nucleotide changes, revealing low levels of genetic diversity within populations and high levels of genetic differentiation between populations. Private *B. pfeifferi* C01 haplotypes that were identified were haplotype 2, haplotype 3, haplotype 5, haplotype 7, haplotype 11, haplotype 12, haplotype 13, haplotype 14, haplotype 15, haplotype 16 and haplotype 17. One *B. pfeifferi* C01 haplotype (haplotype 1) spanned across Kenya and Uganda (It was the most shared haplotype. Other shared haplotypes were haplotype 4, haplotype 6 and haplotype 10. Tanzanian snails exhibited unique haplotypes that were only identified in snails from that country (haplotypes 2,3 and 14) (Figure 26).

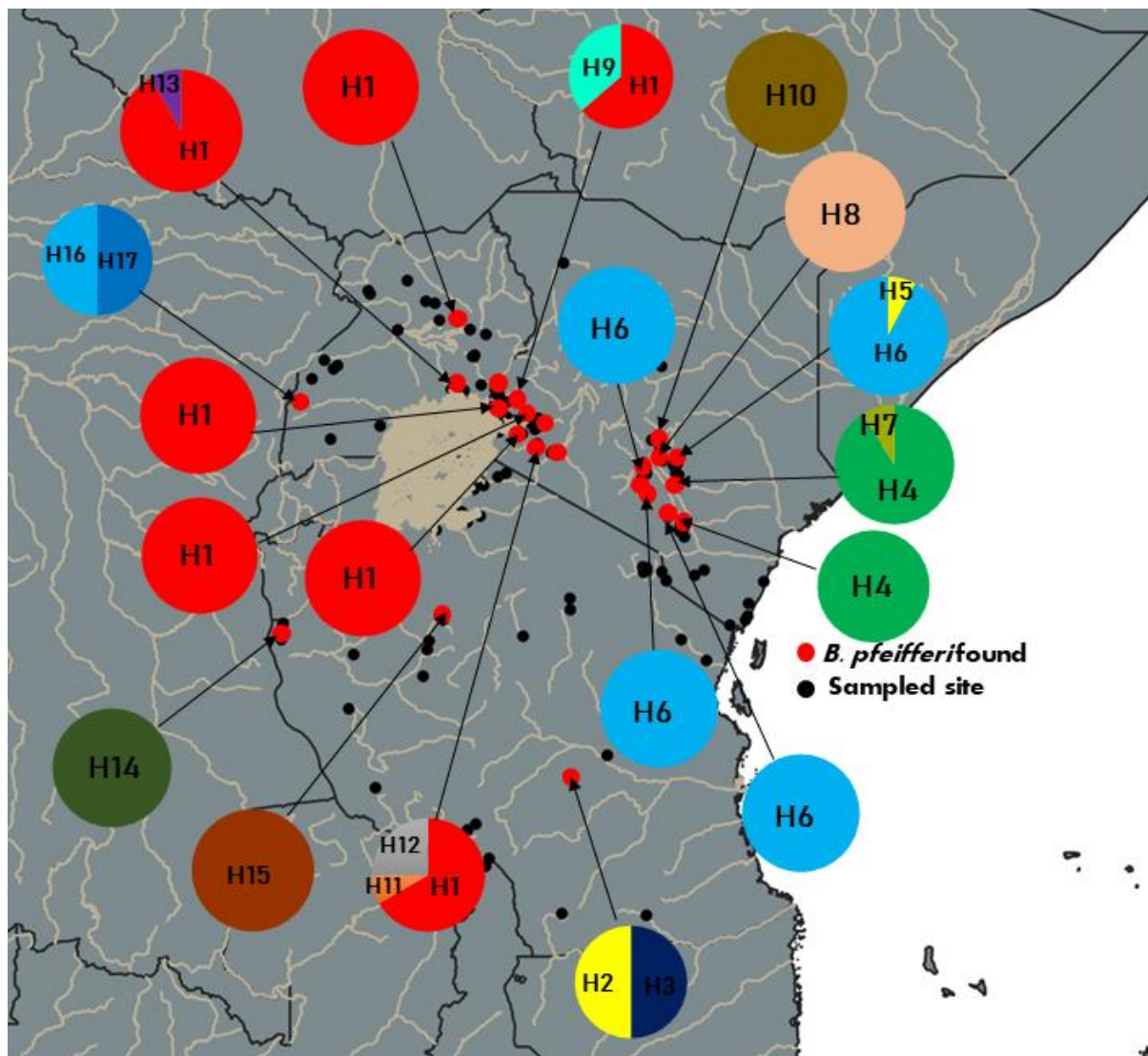


Figure 26: Map of East Africa showing the distribution of 17 *B. pfeifferi* C01 haplotypes.

PopArt software version 1.7 was used to generate a median joining network that revealed the relationship that exists between the different types of haplotypes.

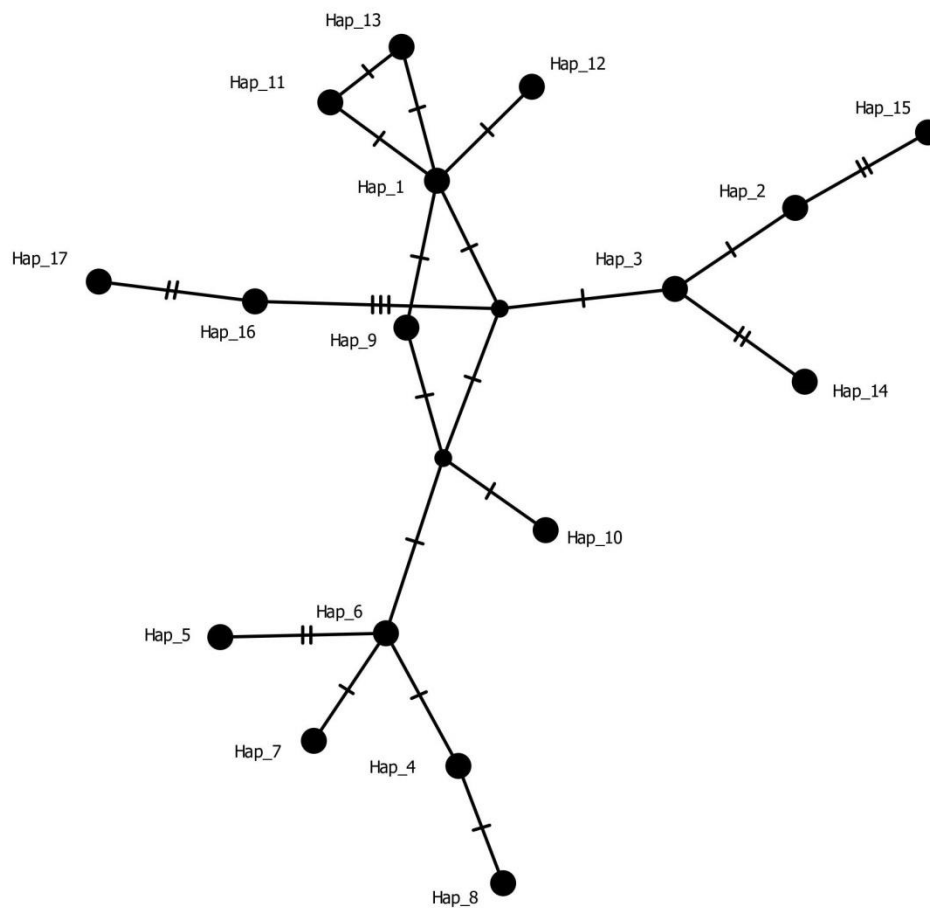


Figure 27: Median joining haplotype network generated using CO1 sequences from *B. pfeifferi* individuals collected from East Africa's River systems.

A total of 21 'Nilotic Species Complex' CO1 haplotypes with a haplotype diversity of 0.8974 and nucleotide diversity of 0.01754 were identified. All the 21 haplotypes were private haplotypes. However, there were haplotypes that were mixed at two sites. Kibimba site had two private haplotypes (haplotypes 2 and 3). Kole site had two private haplotypes (haplotypes 4 and 5). Masindi Port site had four private haplotypes (haplotypes 7, 8, 9 and 10). Ntoroko site had 3 private haplotypes (haplotypes 12 and 13). Lubiri site had 7 private haplotypes (haplotypes 14, 15, 16, 17, 18, 19 and 20).

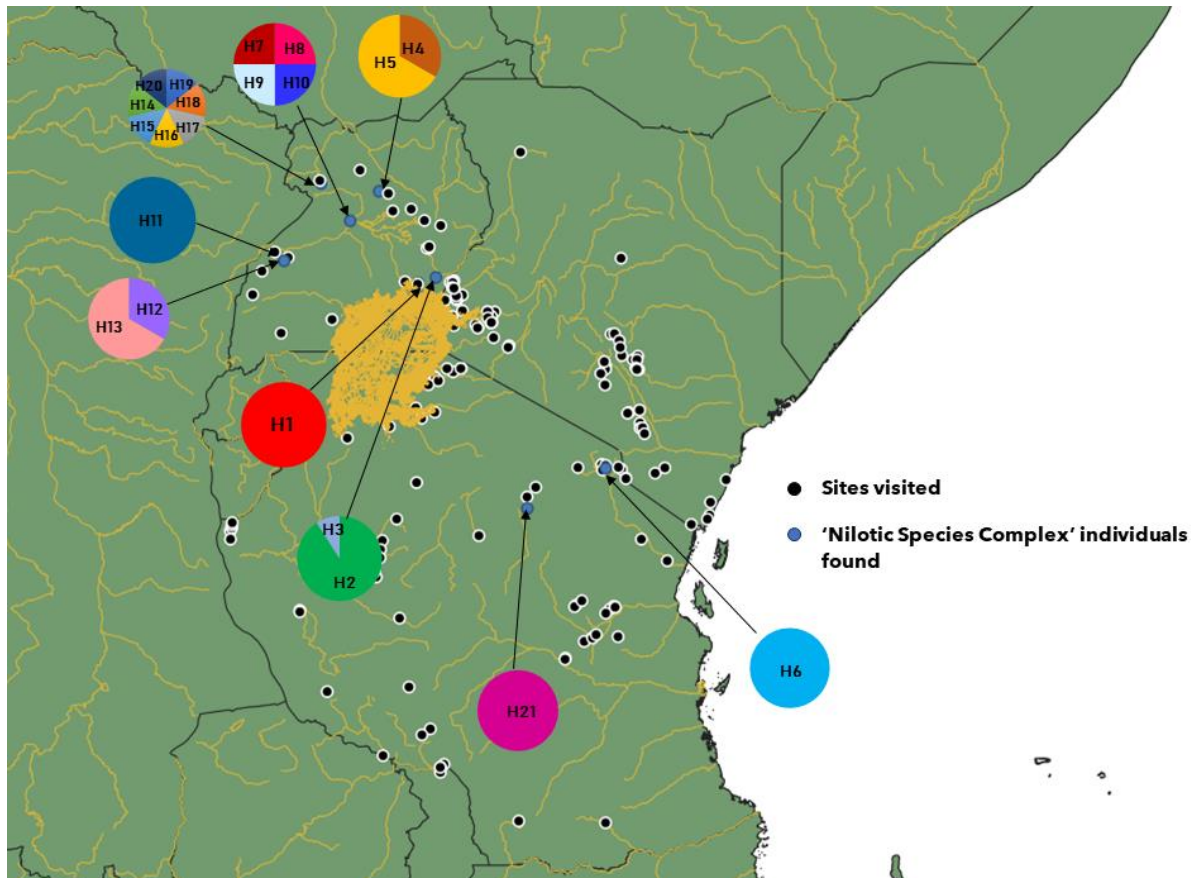


Figure 28: Map of East Africa showing the distribution of CO1 haplotypes belonging to *Biomphalaria* individuals belonging to the 'Nilotic Species complex'.

PopArt software version 1.7 was used to generate a median joining network that revealed the relationship that exists between the different types of haplotypes.

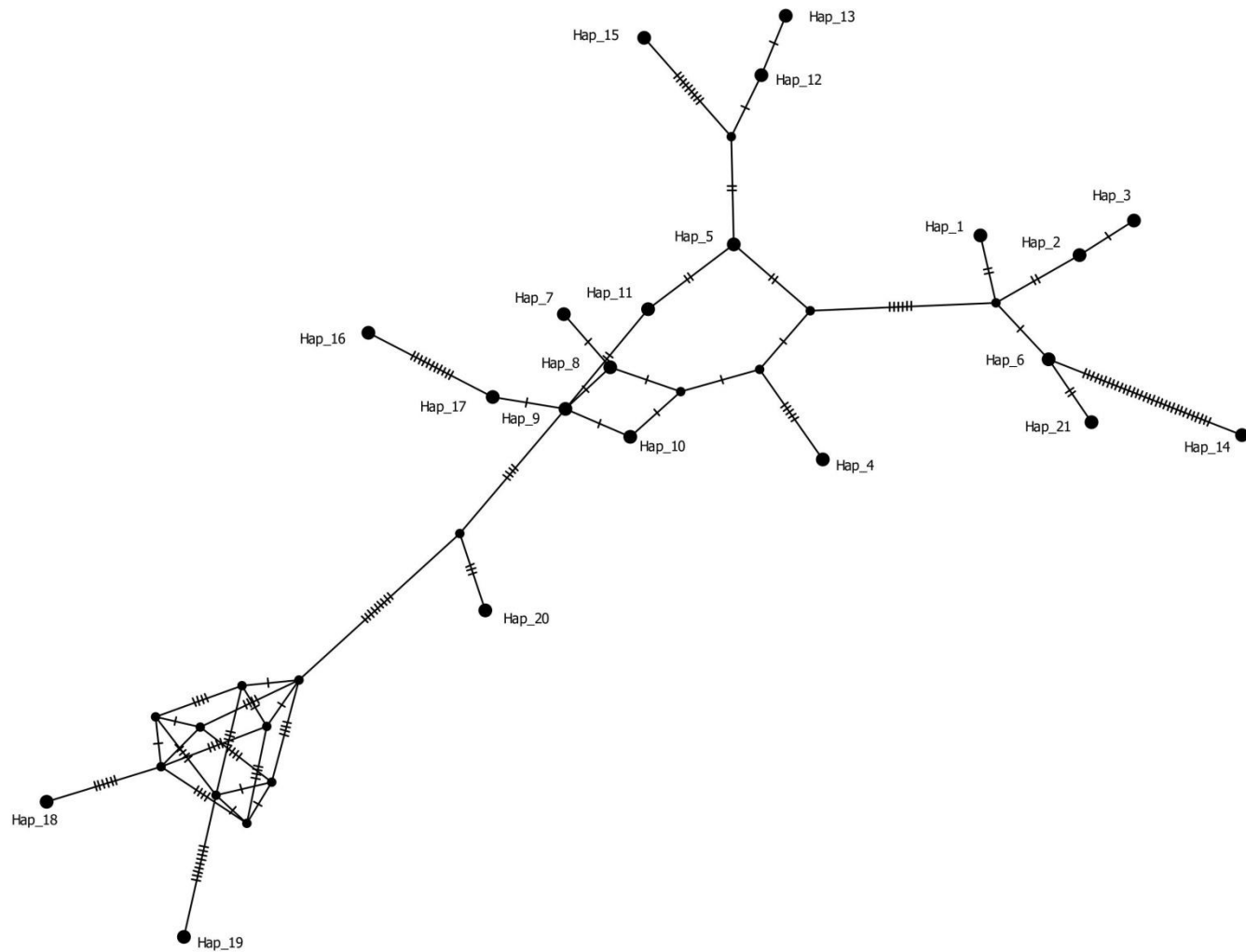


Figure 29: Median joining haplotype network generated using CO1 sequences from 'Nilotic Species Complex' individuals collected from East Africa's River systems.

3.2.3 ITS-2 haplotypes and networks

DnaSP6 software was used to generate haplotype data information. As for ITS-2, 27 *B. pfeifferi* haplotypes with a haplotype diversity of 0.4706 and nucleotide diversity of 0.00222 were identified. There were shared and private haplotypes with haplotype 2 being the most shared haplotype and haplotypes 1 and 17 being the other shared haplotypes. Private haplotypes in Kenya were haplotypes 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 22 and 23. Private haplotypes in Uganda were haplotypes 21, 25, 26 and 27. Private haplotypes in Tanzania were haplotypes 19, 20 and 24 (Figure 30).

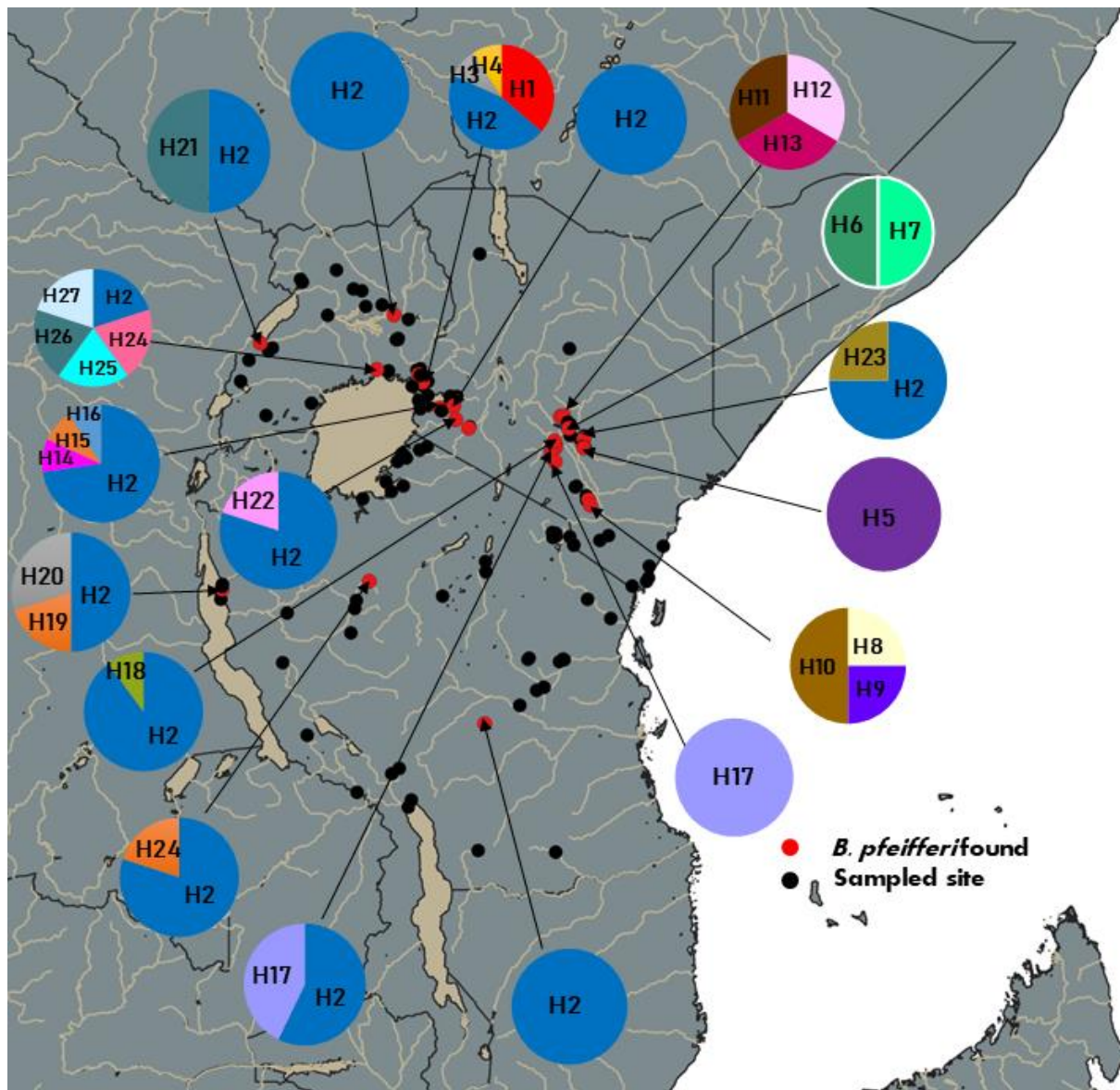


Figure 30: Map of East Africa showing the distribution of *B. pfeifferi* ITS-2 haplotypes.

B. pfeifferi ITS-2 haplotype network shows that haplotype 2 is an integral haplotype directly connected to most of the other haplotypes (Figure 31).

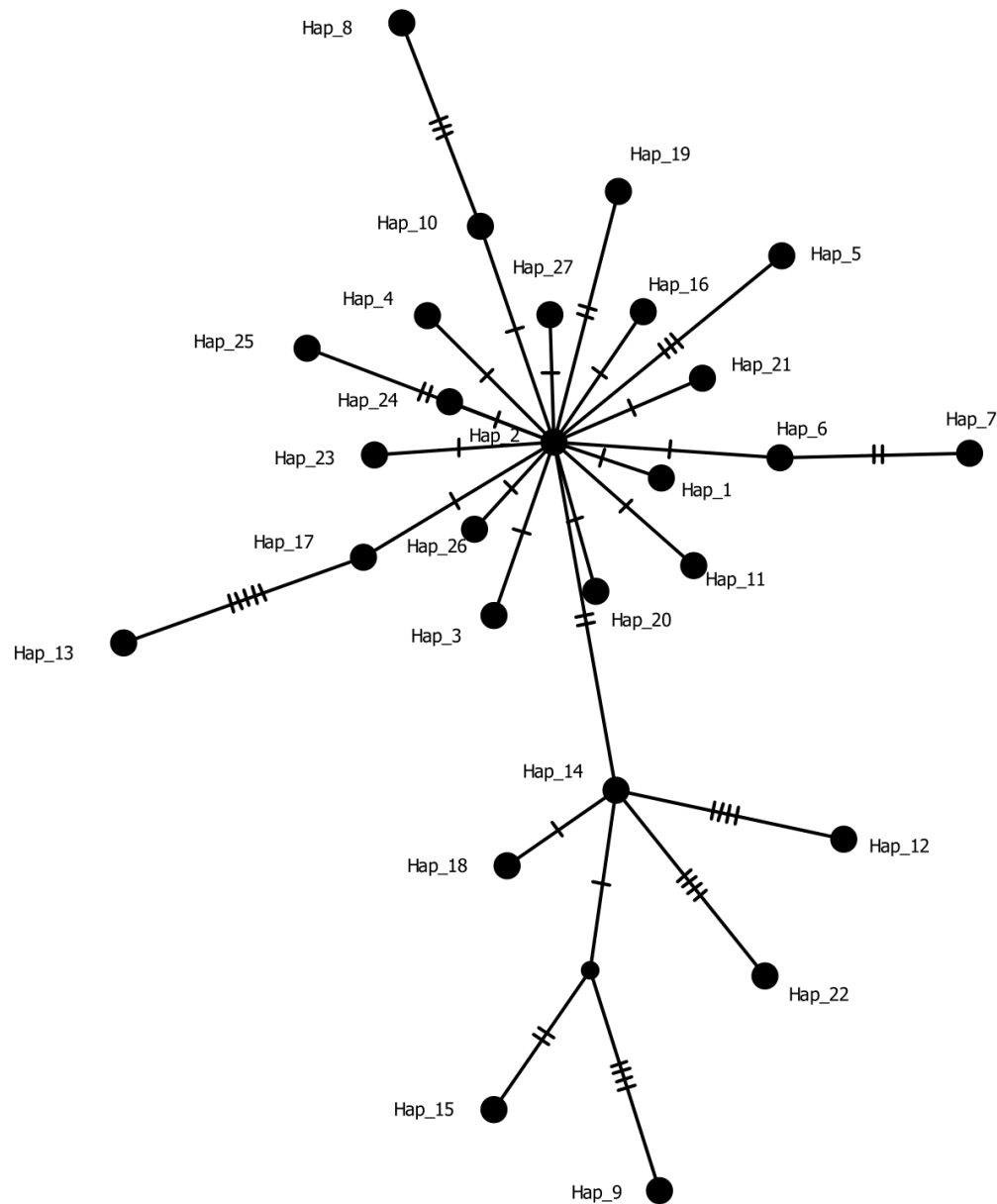


Figure 31: Median joining haplotype network generated using ITS-2 sequences from *B. pfeifferi* individuals collected from East Africa's River systems.

A total of 23 ITS-2 haplotypes were identified for *Biomphalaria* individuals belonging to the 'Nilotic Species Complex' with a haplotype diversity of 0.9505 and nucleotide diversity of 0.00831 (Figure 32). Shared haplotypes were haplotypes 4, 6 and 16 with the rest of the haplotypes being private haplotypes restricted to single sites.

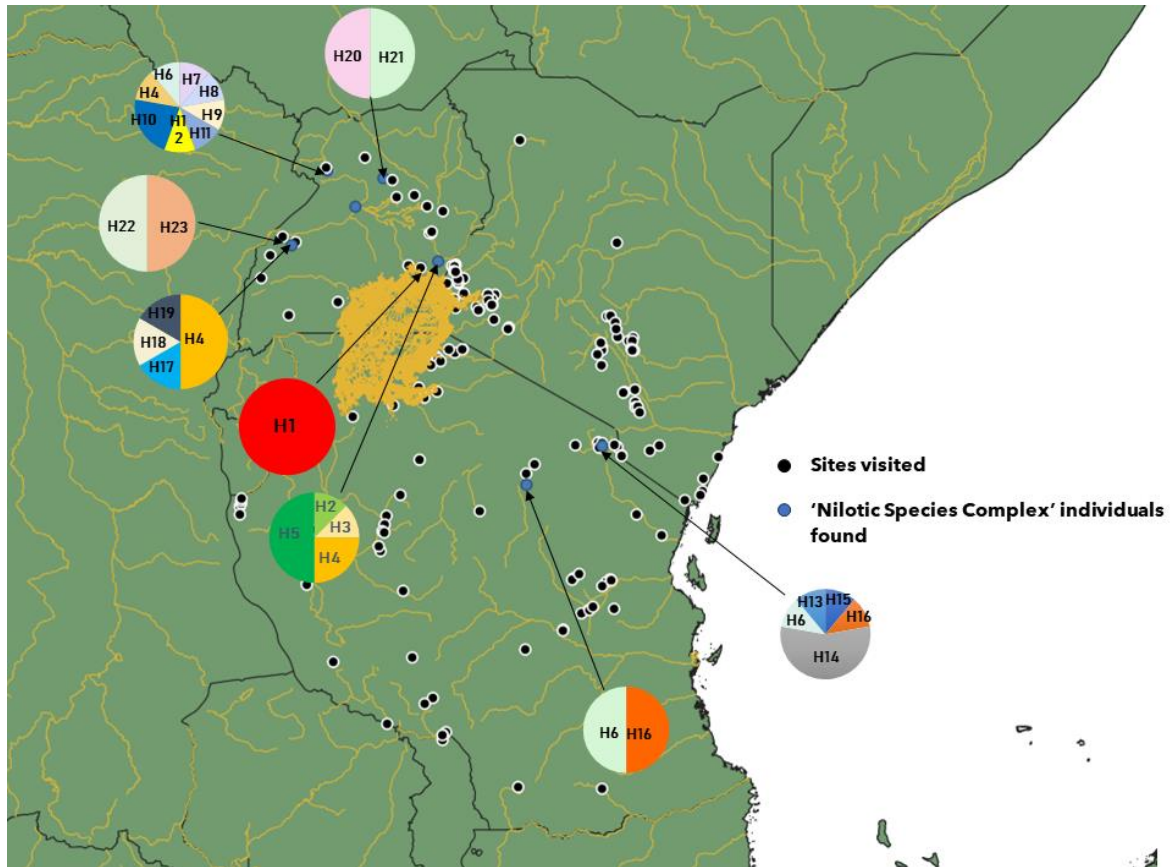


Figure 32: Map of East Africa showing the 23 'Nilotic Species Complex' ITS-2 haplotypes.

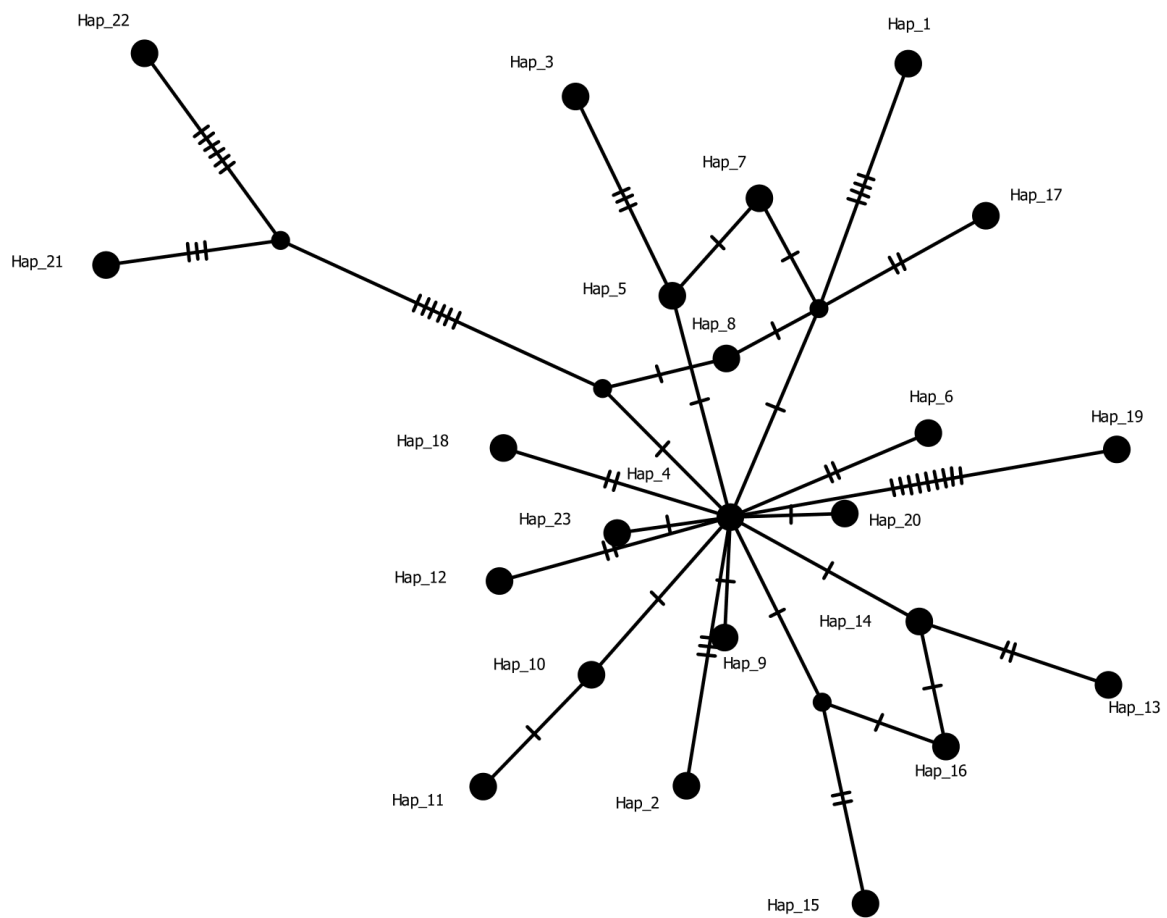


Figure 33: Median joining haplotype network generated using ITS-2 sequences from *Biomphalaria* individuals belonging to the 'Nilotic Species Complex' collected from East Africa's River systems.

3.3 Genetic variation analysis

3.3.1 Genetic variation analysis of *B. pfeifferi* populations

Measures of genetic variation were calculated by the use of the AMOVA statistical method. Analysis of Molecular Variance (AMOVA) for *B. pfeifferi* CO1 data revealed low genetic variation within populations (5.86%) and a high genetic differentiation among populations (94.14%) as confirmed by a high F_{ST} value of 0.94140 (Table 2).

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation
Among populations	22	223.340	1.05117 Va	94.14
Within populations	199	13.020	0.06543 Vb	5.86
Total	221	236.360	1.11660	

Table 2: Analysis of molecular variance (AMOVA) results for *B. pfeifferi* CO1 data, within and among populations.

Analysis of Molecular Variance (AMOVA) for *B. pfeifferi* ITS-2 data revealed low genetic variation within populations (4.83%) and a high genetic differentiation among populations (95.17%) as confirmed by a high F_{ST} value of 0.84131 (Table 3).

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation
Among populations	91	136.292	1.49771 Va	95.17
Within populations	15	48.391	0.26081 Vb	4.83
Total	106	236.360	1.11660	

Table 3: Analysis of molecular variance (AMOVA) results for *B. pfeifferi* ITS-2 data, within and among populations.

Analysis of Molecular Variance (AMOVA) for *B. pfeifferi* CO1 data revealed low genetic variation within populations (4.95%) and a high genetic differentiation (59.37%) among populations within the three countries (Kenya, Uganda and Tanzania) and attested by an F_{ST} value of 0.75053 (Table 4).

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation
Among countries	2	47.080	0.33962 Va	35.68
Among populations, within countries	20	176.260	0.91752 Vb	59.37
Within populations	199	13.020	0.06543 Vc	4.95
Total	221	236.360	1.32257	

Table 4: Analysis of molecular variance (AMOVA) results for *B. pfeifferi* C01 data, among populations within countries.

Analysis of Molecular Variance (AMOVA) for *B. pfeifferi* ITS-2 data revealed low genetic variation within populations (9.26%) and a high genetic differentiation (50.82%) among populations within the three countries (Kenya, Uganda and Tanzania) as confirmed by a high F_{ST} value of 0.74532 (Table 5).

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation
Among countries	1	159.675	5.57178 Va	39.92
Among populations, within countries	2	147.422	5.96958 Vb	50.82
Within populations	20	927.778	48.63889 Vc	9.26
Total	23	1279.875	60.32257	

Table 5: Analysis of molecular variance (AMOVA) results for *B. pfeifferi* ITS-2 data, among populations within countries.

3.3.2 Genetic variation analysis of 'Nilotic Species Complex' populations

Analysis of Molecular Variance (AMOVA) for 'Nilotic Species Complex' COI data revealed moderate genetic variation within populations (28.78%), a moderate level genetic differentiation among populations within groups (49.74%) and a low genetic variation among groups of populations (21.48%) with a high F_{ST} value of 0.71216 (Table 6).

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation
Among groups	1	68.681	1.43743 V_a	21.48
Among populations within groups	7	143.142	3.32926 V_b	49.74
Within populations	47	90.552	1.92664 V_c	28.78
Total	55	302.375	6.69332	

Table 6: Analysis of molecular variance (AMOVA) results for 'Nilotic Species Complex' COI data.

Analysis of molecular variance (AMOVA) results for 'Nilotic Species Complex' ITS-2 data revealed high genetic variation within populations (72.17%) and a low genetic variation among populations (27.83%) with a low F_{ST} value of 0.27827 (Table 7).

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation
Among populations	6	28.577	0.63171 V_a	27.83
Within populations	30	49.153	1.63843 V_b	72.17
Total	36	77.730	2.27014	

Table 7: AMOVA (Analysis of molecular variance) results for 'Nilotic Species Complex' ITS-2 data, within and among populations.

3.4 DISCUSSION

3.4.1 Phylogeography of African *Biomphalaria* snails with a focus on East Africa's River systems

In our analyses, *Biomphalaria camerunensis* takes a basal position among African *Biomphalaria* species. This suggests that *B. camerunensis* is the oldest *Biomphalaria* species on the African continent. All *B. pfeifferi* sequences in our analysis cluster together. This agrees with a study by Jorgensen *et al.* (2007) that averred that *B. pfeifferi* snails are monophyletic. On the other hand, our 'Nilotic Species Complex' sequences intermingle on the phylogenetic trees. From analysis of the sequences, we deduce that members of the 'Nilotic Species Complex' are not monophyletic. In both CO1 and ITS-2 phylogenetic trees *B. choanomphala* group and *B. sudanica* group members cluster with other members of the 'Nilotic species complex.' The 'Nilotic species complex' refers to a group of *Biomphalaria* species that radiated in the Lake Victoria basin, in the Albertine Rift Valley lakes and further along River Nile. Members of the 'Nilotic species complex' are *B. smithi*, *B. sudanica*, *B. choanomphala*, *B. stanleyi* and *B. alexandrina* (Jorgensen *et al.*, 2007). In our study, *B. angulosa* takes a basal position to the 'Nilotic species complex.' This is consistent with a study by DeJong *et al.* (2001) that suggested that the 'Nilotic species complex' radiated from *B. angulosa*.

Phylogenetic analyses of *B. pfeifferi* sequences reveals that *B. pfeifferi* snails from Madagascar are probably the oldest *B. pfeifferi* snails in Africa. This agrees with a phylogenetic study by DeJong *et al.* (2003) that asserted that *B. pfeifferi* haplotypes from South Africa and Madagascar are the most ancestral on the African continent.

Among our East Africa's *B. pfeifferi* sequences, Ugandan sequences collected from Ntoroko, at the border of Uganda and the Democratic Republic of Congo take a position closer to sequences from Sudan and Zimbabwe; followed by our sequences from Tanzania. This could suggest that *B. pfeifferi* snails from Uganda are the oldest followed by *B. pfeifferi* from Tanzania. According to our analysis, it could be inferred that Kenyan *B. pfeifferi* snails are the youngest in East Africa. Previous phylogenetic studies on *B. pfeifferi* snails on the African continent affirm that *B. pfeifferi* took a West to East direction on its colonisation of the African continent, therefore the snail arrived first in Uganda before it arrived in Tanzania and Kenya (Uganda is more western in longitude compared to Tanzania and Kenya).

It can be deduced from our phylogenetic trees that geographical distance plays a role in bringing about genetic differentiation among *Biomphalaria* populations in East Africa's River systems. Sequences of *Biomphalaria* populations that were found at sites close to each other geographically took close positions to each other in the phylogenetic trees. Sequences of snails from Western Kenya took close positions to each other in the phylogenetic trees. Sequences of snails from Eastern Province, Kenya took close positions to each other in the phylogenetic trees, Sequences of snails from Western Uganda took closer positions to each other in the phylogenetic trees. Sequences of snails from Northern Tanzania took close positions to each other in the phylogenetic trees. Sequences of snails from Southern Tanzania took close positions to each other in the phylogenetic trees. There

are studies that have shown that geographical distance is likely to prevent gene flow and therefore increase levels of genetic differentiation among distantly located *Biomphalaria* populations (Campbell *et al.*, 2010; Mavarez *et al.*, 2002; Langand *et al.*, 1999).

There is a need to reclassify African *Biomphalaria* snails. An earlier classification by Mandahl (1957), categorises *B. camerunensis* as part of the *B. sudanica* group yet in our study *B. camerunensis* does not cluster with *B. sudanica* group. Furthermore, a classification by DeJong *et al.* (2001), categorises *B. stanleyi* as not being part of the 'Nilotic species complex' yet in our study *B. stanleyi* clusters with members of the 'Nilotic species complex' (*B. sudanica*, *B. choanomphala*, *B. smithi* and *B. alexandrina*). Geographical distance also proves to be a major factor contributing to increased levels of genetic differentiation among *Biomphalaria* populations (there is a correlation between genetic distance and geographical distance).

3.4.2 The Population genetic structure of *Biomphalaria* snails in East Africa's River systems

Genetic diversity in populations is dependent on an interplay between factors that generate local genetic differentiation and factors that generate genetic homogeneity (Wethington *et al.*, 2007). *B. pfeifferi* populations studied herein were characterised by low intrapopulation diversity, high levels of population genetic structure, suggestive of low levels of gene flow and high levels of inbreeding as deduced from our molecular data (both CO1 and ITS-2 data). Low intrapopulation genetic diversity has also been reported in *B. pfeifferi* populations from Zimbabwe (Mutsaka *et al.*, 2020). In the case of the 'Nilotic species complex', there was moderate to high levels of intrapopulation genetic diversity and high levels of genetic differentiation. High F_{ST} values were reported for *B. pfeifferi* populations and moderate F_{ST} values reported for members of the 'Nilotic Species Complex.' High intrapopulation genetic diversity has also been reported in the study of *B. choanomphala* snails from Lake Victoria (Standley *et al.*, 2014). In the past, high F_{ST} values have also been reported in a population genetic structure study of *B. pfeifferi* populations from Cameroon, suggestive of low gene flow among the populations (Tchami *et al.*, 2020). The results herein corroborate with studies of *B. pfeifferi* in Madagascar where low intrapopulation genetic diversity and high interpopulation genetic variation was reported (Charbonnel *et al.*, 2002a). This study also agrees with a study by DeJong *et al.* (2001) that showed a genetic difference of 0.29–0.50% among seven *B. pfeifferi* populations. This study also agrees with a study by Bandoni *et al.* (1990) on 12 *B. pfeifferi* populations from Kenya that revealed a marked deficiency of heterozygotes in the populations. It was evident that the Kenyan *B. pfeifferi* populations indulged in non-random mating (self-fertilization).

Evolutionary forces, biotic factors and abiotic factors are likely to explain the differences in intra and interpopulation genetic variation among the different *Biomphalaria* species. Factors that affect the population genetic structuring process of *Biomphalaria* snails include the type of fertilisation preferred by the snails, parasitism, habitat stability, genetic drift and gene flow (Christiansen, 2008; Sandland *et al.* 2007; Charbonnel *et al.* 2005). Previous studies suggest that *B. pfeifferi* snails prefer self-fertilization to cross fertilization with the former contributing significantly to deficiency of heterozygotes in most *B. pfeifferi* populations (Bandoni *et al.*, 1990; Mimpfound & Greer, 1990). *B. pfeifferi* snails prefer self-

fertilization as a reproductive assurance strategy because they live in habitats where migration is rare and extinction is frequent (Charbonnel *et al.*, 2005). On the other hand, members of the 'Nilotic species complex' have not been reported to favour either self-fertilisation or cross fertilisation, implying that the snails can equally use either mode of fertilisation for reproduction (random mating) (Standley *et al.*, 2014).

Ecological factors such as drought and flooding have also been reported to play an important role in creating bottlenecks, recolonisation events and possible extinction of *Biomphalaria* snails, consequentially leading to the genetic structuring process of populations (Mutsaka *et al.*, 2020). Genetic drift and divergent selection are the major evolutionary forces that promote genetic structuring process of populations whereas gene flow as an evolutionary force homogenizes sub-populations (Jones & Wang, 2012). A high level of population genetic differentiation among *Biomphalaria* populations suggests low gene flow.

Parasitism potentially contributes to the genetic structuring process of *Biomphalaria* snails in East Africa's River systems. The results from this study corroborate with results by Standley *et al.* (2014), on the population genetic studies of *B. choanomphala* that revealed relatively high levels of intrapopulation genetic diversity could be attributed to the role that parasites play in localised genetic structuring processes of their intermediate hosts. Previous studies by Webster *et al.* (2007) and Sandland *et al.* (2007) assert that parasitism influences the population genetic structuring processes of snail intermediate hosts.

Habitat stability is also another factor that potentially has a role to play in the genetic structuring process of *Biomphalaria* populations (Tian-Bi *et al.*, 2013). *B. pfeifferi* snails mostly inhabit unstable habitats that are prone to destruction by floods and droughts whereas members of the 'Nilotic species complex' mostly inhabit stable habitats that are not adversely affected by extreme weather conditions. Additionally, most *B. pfeifferi* populations live in geographically isolated habitats and because of this, geneflow tends to be restricted, leading to mating involving genetically identical individuals (biparental inbreeding). Members of the 'Nilotic species complex' inhabit the Albertine Rift Valley Lakes and the Lake Victoria basin ecosystems which are relatively stable and not adversely affected by extreme weather conditions (Jorgensen *et al.*, 2007). In addition, the relative stable habitats inhabited by *B. choanomphala* group members, *B. sudanica* group members and *B. stanleyi* group members promote incidences of geneflow which consequentially leads to an increase in genetic diversity within the snail populations. The contrast in habitat stability probably explains the low intrapopulation genetic diversity in *B. pfeifferi* group populations and the relatively high genetic diversity in the 'Nilotic species complex' populations.

Geographical isolation and geographical distance can potentially contribute to the genetic structuring process of *Biomphalaria* populations as revealed by Bandoni *et al.* (1990). In a study on 12 *B. pfeifferi* populations from Kenya, marked deficiency of heterozygotes was reported but the genetic differentiation among populations was associated with the geographical distance among the populations. Populations that were geographically closer

to each other had relatively similar genetic structure, stressing the importance of geographical distance in facilitating gene flow.

3.5 CONCLUSION

In conclusion, population genetic analysis revealed that *B. pfeifferi* snails are strictly monophyletic whereas members of the 'Nilotic Species Complex' intermingle on the phylogenetic trees. However, a deduction of the 'Nilotic Species Complex' individuals can be done into three groups namely '*B. choanomphala* type' group, '*B. sudanica* type' group and the '*B. stanleyi* type' group. *B. camerunensis* takes a basal position in both CO1 and ITS-2 trees, suggesting and confirming that it is the oldest member of African *Biomphalaria* snails. Individuals from locations that are closer geographically appear closer to each other on the phylogenetic trees confirming the influence that geography has on genetic diversity. As for haplotypes, there was a clear geographical clustering of haplotypes with 18 *B. pfeifferi* CO1 haplotypes and 20 'Nilotic Species Complex' CO1 haplotypes being identified. As for ITS-2 sequence data, there was identification of 27 *B. pfeifferi* ITS-2 haplotypes and 23 'Nilotic Species Complex' ITS-2 haplotypes. Significant divergent haplotypes were not found within the same population, suggesting very low to moderate incidents of gene flow. Genetic variation within *B. pfeifferi* populations was low suggestive of inbreeding whereas genetic variation within 'Nilotic Species Complex' populations was moderate suggestive of incidences of cross breeding and gene flow. As for genetic differentiation among populations, there were high levels of genetic population differentiation among *B. pfeifferi* populations and moderate levels for 'Nilotic Species Complex' populations.

CHAPTER FOUR: GEOGRAPHICAL DISTRIBUTION OF *BIOMPHALARIA* SNAILS IN EAST AFRICA'S RIVER SYSTEMS

ABSTRACT

There is limited information on the distribution of *Biomphalaria* snails in East Africa's River systems. This study assessed the incidence and geographical distribution of *Biomphalaria* snails in East Africa's River systems (Kenya, Uganda, and Tanzania). Malacological surveys were conducted at a total of 172 sites including streams, rivers, dams, springs, and irrigation schemes over a 3-year period (2018 to 2020). Geospatial, ecological, and physicochemical information were recorded for the sites visited. *Biomphalaria* snails were identified using the CO1 gene as the molecular marker. *Biomphalaria* snails were found at 18% (31/172) of the total sites sampled with *B. pfeifferi* snails being found at 23/31 sites whereas members of the 'Nilotic Species complex' being found at 8/31 sites. Streams proved to be the habitats most preferred by the snails (61.3% of all the sites where the snails were found were streams), followed by rivers (22.58%), irrigation schemes (9.68%) and dams (6.45%) with snail abundance increasing with increase in temperature and decrease in water depth. In Kenya the snails were found in the Lake Victoria basin, Mwea Irrigation Scheme, and the former Eastern Province. In Uganda, the snails were found in Masindi District, Kole District, Pakwach District, Ntoroko District, Kyenjojo District and Soroti District. In Tanzania, the snails were found in Kilimanjaro Region, Manyara region, Iringa region, Tabora region and Kigoma region. This information on the distribution of *Biomphalaria* snails in East Africa's River systems can aid in developing better prevention and control strategies for schistosomiasis.

4.1 INTRODUCTION

Schistosomiasis is a neglected tropical disease caused by flatworms of the genus *Schistosoma*. The disease is ranked second to malaria in terms of tropical diseases of public health importance (Colley *et al.*, 2014). It is estimated that about 1 billion people globally are at risk of being infected with the disease and about 250 million people are currently infected (Gryseels, 2012). 200,000 deaths are attributable to the disease annually (Colley *et al.*, 2014). In East Africa, it is estimated that about 40 million people are infected with schistosomiasis and about 140 million people are at risk of infection (Aula *et al.*, 2021). The disease has debilitating effects on individuals because it renders them unproductive due to malaise and disability. In particular, the disease is catastrophic to school aged children because it impedes them from attending school due to physical incapacity associated with the disease, thus jeopardising their chances of having a brighter future. There are two forms of schistosomiasis, urogenital schistosomiasis caused by *S. haematobium*, and intestinal schistosomiasis caused by *S. mansoni* and *S. japonicum* (Colley *et al.*, 2014). Other human *Schistosoma* parasites albeit not significant from a public health perspective include *S. intercalatum*, *S. mekongi* and *S. guineensis* (Gryseels, 2012). *Schistosoma mansoni* is responsible for most cases of human schistosomiasis in Africa and the Americas; it is solely responsible for 83 million out of an estimated total 250 million global schistosome infections (Morgan *et al.*, 2005).

Snail intermediate hosts are important because they support the transformation of *Schistosoma* parasites into infective stages (Zeng *et al.*, 2017). Snail intermediate hosts for *S. mansoni* belong to the genus *Biomphalaria*. In East Africa, there are several species of the genus *Biomphalaria* including *B. sudanica*, *B. choanomphala*, *B. smithi*, *B. stanleyi*, *B. angulosa* and *B. pfeifferi* (Standley *et al.*, 2012a).

B. pfeifferi, *B. sudanica* and *B. stanleyi* have been identified from Lake Albert (Rowel *et al.*, 2015) while *B. sudanica* and *B. choanomphala* have been identified from Lake Victoria (Standley *et al.*, 2014; Standley *et al.*, 2011). *B. sudanica* and *B. smithi* have been found from Lake Edward (Mandahl, 1957). *Biomphalaria* snails, although not identified to the species level have been identified at higher altitudes of Mount Elgon, Rwenzori Mountains and Fort Portal crater lakes (Stanton *et al.*, 2017). *B. sudanica* snails have also been found in Northern and North-eastern parts of Uganda (Habib *et al.*, 2021).

Previous studies of *Biomphalaria* snails in East Africa's River systems have found *Biomphalaria* sp. snails in Uganda at Lwampanga, Masindi Port, Muzizi, Ngamilajojo, Butiaba and Ntoroko (Lake Albert) (Jorgensen *et al.*, 2007). *Biomphalaria* sp. snails have also been found in Northern and North-eastern parts of Uganda (Habib *et al.*, 2021). In Kenya, *B. pfeifferi* snails have been found at Muthamo seepage, Matingani seepage, Mbondoni dam, Kangonde dam, Mwea East, Mwea West, Onsando dam, Grogan Canal, Kamayoga stream, Asawo stream, Kwahoma stream and Martin's drain (Bandoni *et al.*, 1990). Malacological surveys by Opisa *et al.* (2011), at informal settlements of Kisumu City, Kenya identified two *Biomphalaria* species, *B. sudanica* and *B. pfeifferi*. *B. pfeifferi* snails were also found in Kenya, at the Mwea irrigation scheme, Asawo stream and Mukou stream by Mutuku *et al.* (2020). A study by Buddenborg *et al.* (2017) identified and collected *B. pfeifferi* from Kasabong stream, Asembo village (Western Kenya). *B. pfeifferi* snails have

also been reported from Kenya at Kibwezi (Jorgensen *et al.*, 2007) as well as Chebunyo dam (Bomet County) and Churo pond (Kirinyaga County) (Bandoni *et al.*, 2000). Furthermore, *B. pfeifferi* snails have also been collected from Asawo River and Kasabong stream, in Western Kenya (Lu *et al.*, 2016). In Tanzania, *B. pfeifferi* snails have been found at the southern part of the country, around Gombe National Park (Bakuza *et al.*, 2017). *Biomphalaria* sp. snails have been identified in the Northern part of Tanzania (in Babati District and Lower Moshi irrigation scheme) by Lydig (2009) and Kisanga (1991) respectively. *B. pfeifferi* snails have also been found in Kilombero District, Tanzania (Utzinger & Tanner, 2000). Previous studies on the distribution of *Biomphalaria* snails in East Africa's River Systems (Kenya, Uganda and Tanzania) have not covered expansive geographical areas. The abundance of *Biomphalaria* snails in any geographical location is important because the greater the abundance of snail intermediate hosts in any location, the higher the probability that human beings will come in contact with *S. mansoni* and be infected (Bakuza *et al.*, 2017).

Intestinal schistosomiasis has been reported to be endemic in several localities in East Africa (Aula *et al.*, 2021). However, there has been little information on the role that *Biomphalaria* snails play in transmission of schistosomiasis, outside Lake Victoria and Lake Albert. An increase in schistosome infections in snail intermediate hosts, leads to contamination of water bodies with active cercariae thus meaning that human beings become at more risk of being infected with the parasites. Some of the localities and regions where intestinal schistosomiasis has been reported to be endemic, with little to no reports on snail intermediate hosts include Lango region (Adriko *et al.*, 2018), West Nile region (Imran, 2014), Soroti District and Kilimanjaro region (Ejotre *et al.*, 2014). There is therefore a need to extensively establish the distribution of *Biomphalaria* snails in East Africa's River systems, because of limited information about their distribution and their significance as an important intermediate host in the transmission of schistosomiasis. Understanding distribution of *Biomphalaria* snails in East Africa's River systems would influence decision making as far as development of effective and integrated strategies to combat transmission of human intestinal schistosomiasis is concerned.

This study aimed to assess the incidence of *Biomphalaria* snails and their current geographical distribution in East Africa's River systems as well as seeking to determine habitat types and physicochemical and ecological parameters associated with the snails.

4.2 RESULTS

4.2.1 Potential distribution of *Biomphalaria* snails in Africa as determined by MaxEnt

MaxEnt analysis was used to predict the distribution of the *Biomphalaria* snails.

Biomphalaria snails are predicted to be widely distributed in East Africa based on MaxEnt analysis (Figure 34). The dark red colour signifies locations with the highest probability of *Biomphalaria* snails being found; the light red colour signifies locations with the second highest probability of *Biomphalaria* snails being found; the orange colour signifies locations with the third highest probability of *Biomphalaria* snails being found; the dark yellow colour signifies locations with the fourth highest probability of *Biomphalaria* snails being found; the light yellow colour signifies locations with the fifth highest probability of *Biomphalaria* snails being found; the dark green colour signifies locations with the sixth highest

probability of *Biomphalaria* snails being found; the light green colour signifies locations with the seventh highest probability of *Biomphalaria* snails being found. Light blue colour signifies locations with the third least probability of *Biomphalaria* snails being found. Azure blue signifies locations with the second least probability of *Biomphalaria* snails being found. Dark blue signifies locations with the least probability of *Biomphalaria* snails being found.

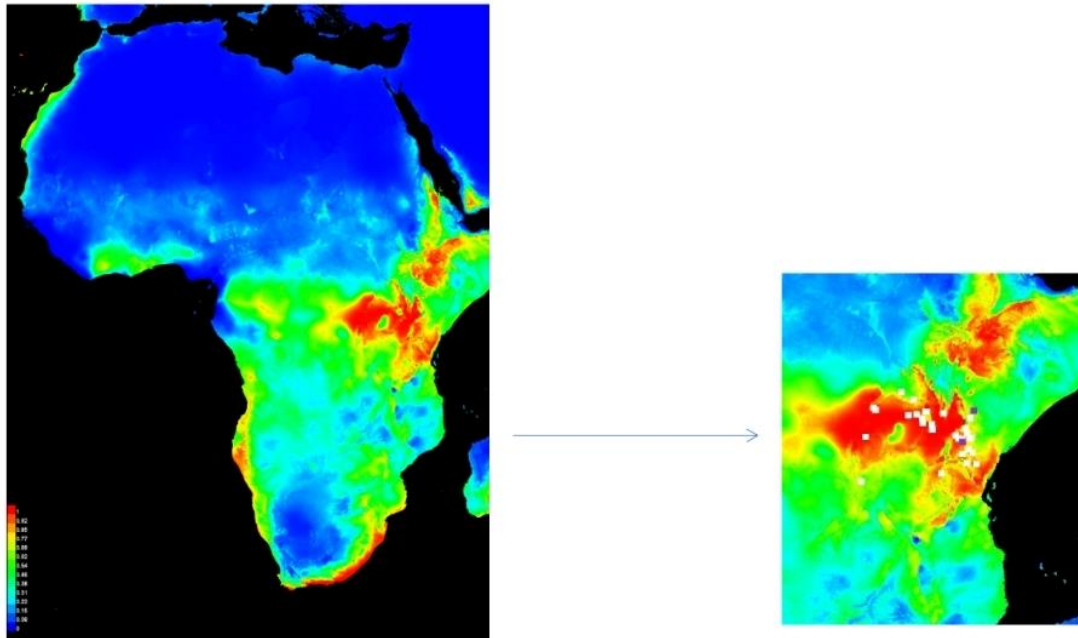


Figure 34: Potential distribution of *Biomphalaria* snails in Africa and East Africa, courtesy of MaxEnt.

4.2.2 Presence and incidence of *Biomphalaria* snails

Malacological surveys were conducted with a total of 172 sites visited. *Biomphalaria* snails were found at 31 out of the 172 surveyed sites (Table 8 and Figure 35). At most sites just a single *Biomphalaria* species was found but there were 3 sites where we found mixed *Biomphalaria* species. At Ntoroko, *B. pfeifferi* and individuals belonging to the 'Nilotic Species Complex' were found; at Kole and Lubiri, a mixture of individuals belonging to the 'Nilotic Species Complex' were found.

Biomphalaria species found in the rivers systems were *B. pfeifferi* (at 23/31 sites) and members of the 'Nilotic Species Complex' (8/31 sites). Streams proved to be the habitats most preferred by the *Biomphalaria* snails (61.3% of all the sites where the snails were found were streams), followed by rivers (22.58%), irrigation schemes (9.68%) and dams (6.45%).

Name of site	Geographic Coordinates	Altitude	Type of habitat	Water Temp (°C)	pH	Water depth (cm)	Water velocity (cm/s)	Soil Type	Veg (Y/N)
Matingani (Kenya)	(-1.164, 38.005)	1184m	Stream	23.2	5.2	23.7	4.3	Clay soil	Y
Kiangangi (Kenya)	(-0.593833, 37.341972)	1365m	Irrigation scheme	24.5	8.2	17.5	6.5	Silt soil	Y
Kitui (Kenya)	(-1.35616667, 38.00611111)	1166m	Stream	20.6	6.4	16.3	12.4	Silt soil	Y
Kalundu (Kenya)	(-1.35616667, 38.00611111)	1216m	Stream	20.4	7.3	23.1	13.5	Sandy soil	Y
Kwase (Kenya)	(-1.29883333, 37.35722222)	1568m	Stream	19.3	6.8	19.3	16.5	Silt soil	Y
Mutanga (Kenya)	(-1.35758333, 37.35472222)	1484m	Stream	25.7	8.2	25.1	18.3	Silt soil	Y
Itua (Kenya)	(-0.62888889, 37.54)	653m	River	25.4	8.3	28.3	38.2	Silt soil	Y
Tulimumbu (Kenya)	(-0.91133333, 37.65833333)	1007m	Stream	21.3	6.8	23.1	16.5	Sandy soil	Y
Musilili (Kenya)	(-1.45023611, 37.2575)	1330m	Stream	24.6	8.3	25.2	18.5	Silt soil	Y
Kakulutuine (Kenya)	(-1.20472222, 37.33055556)	1188m	Stream	25.3	7.6	21.7	15.3	Silt soil	Y
Mukou (Kenya)	(-1.68488889, 37.34472222)	1319m	Stream	25.9	9.2	19.2	19.5	Silt soil	Y
Ambururu (Kenya)	(0.15163889, 34.27916667)	1236m	Stream	23.5	7.5	20.2	26.4	Silt soil	Y
Rakite (Kenya)	(0.32575, 34.19472222)	1285m	Stream	21.2	8.2	23.1	12.4	Silt soil	Y
Asawo (Kenya)	(-0.31817, 35.007)	1232m	River	24.5	6.8	28.6	23.7	Silt soil	Y
Kodumo (Kenya)	(-0.41068, 34.99455)	1532m	Stream	21.2	7.3	29.7	12.9	Silt soil	Y

Kodongo (Kenya)	(-0.444710, 34.681030)	1548	Stream	16.2	6.9	26.7	15.4	Silt soil	Y
Onsando (Kenya)	(-0.71103, 35.0479)	1926m	Dam	25.4	6.7	42.6	4.2	Silt soil	Y
Cheptuyet (Kenya)	(-0.91062, 35.34843)	1914m	Stream	25.3	8.3	27.3	14.1	Silt soil	Y
Walukuba (Uganda)	(0.44258, 33.22391)	1139m	Stream	25.6	7.3	26.3	14.2	Silt soil	Y
Opiyai (Uganda)	(1.70238, 33.62261)	1119m	Spring	25.2	6.2	27.2	12.6	Silt soil	Y
Ntoroko (Uganda)	(1.05375, 30.53696)	631m	River	20.8	8.4	28.6	13.6	Silt soil	Y
Bwawani (Tanzania)	(-7.73513, 35.71792)	1530m	Dam	24.1	8.5	18.6	13.7	Silt soil	Y
Mwamgongo (Tanzania)	(-4.62742, 29.65162)	676m	Stream	25.9	6.8	28.1	13.7	Silt soil	Y
Kabere (Uganda)	(0.41284, 33.49619)	1156m	Stream	21.3	7.2	19.2	7.1	Silt soil	Y
Kibimba (Uganda)	(0.52956, 33.85815)	1080m	Irrigation scheme	24.7	7.3	14.6	11.2	Silt soil	Y
Kole (Uganda)	(2.302313, 32.68811)	1056m	River	21.3	6.3	19.2	6.4	Silt soil	Y
Mabogini (Tanzania)	(-3.40061, 37.36256)	684m	Irrigation scheme	24.3	8.8	23.6	12.3	Silt soil	Y
Masindi (Uganda)	(1.69541, 32.09238)	1034m	River	19.2	6.7	26.4	7.4	Silt soil	Y
Muzizi (Uganda)	(0.87095, 30.72997)	1178m	River	21.1	9.2	21.5	9.6	Silt soil	Y
Ntoroko (Uganda)	(1.05375, 30.53696)	631m	River	20.8	8.4	28.4	10.4	Silt soil	Y
Lubiri (Uganda)	(2.45857, 31.49975)	632m	River	22.1	7.3	19.7	12.5	Silt soil	Y
Babati (Tanzania)	(-4.227588, 35.744211)	979m	River	24.5	8.1	27.2	8.3	Silt soil	Y

Table 8: Table of sites visited where *Biomphalaria* snails were found in East Africa's River systems

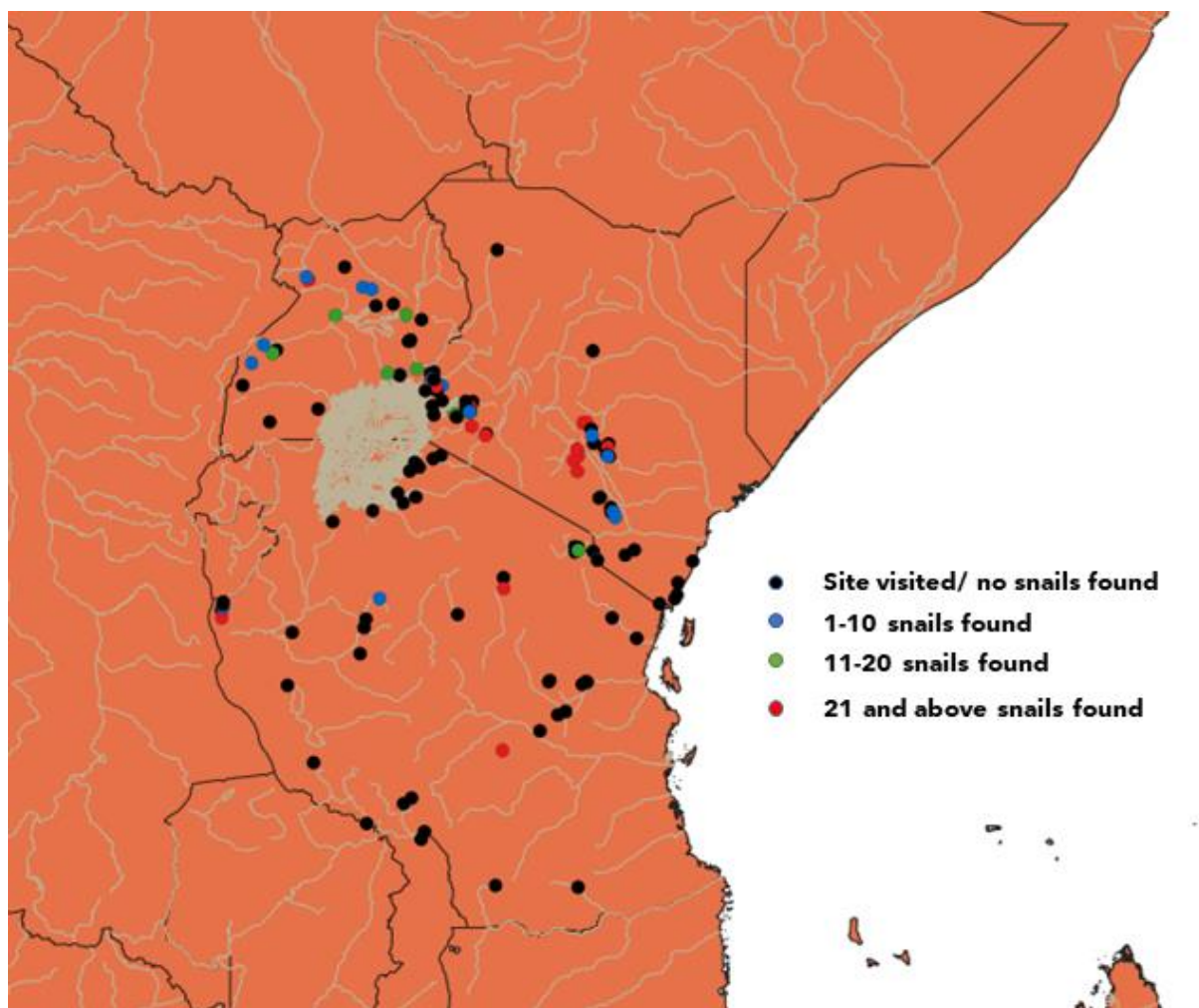


Figure 35: Incidences of *Biomphalaria* snails in East Africa's River systems.

Name of site	Geographic Coordinates	Altitude	Type of habitat	Water Temp (°C)	pH	Water depth (cm)	Water velocity (cm/s)	Soil Type	Veg (Y/N)
Matingani (Kenya)	(-1.164, 38.005)	1184m	Stream	23.2	5.2	23.7	4.3	Clay soil	Y
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Ntoroko (Uganda)	(1.05375, 30.53696)	631m	River	20.8	8.4	28.6	13.6	Silt soil	Y

Bwawani (Tanzania)	(-7.73513, 35.71792)	1530m	Dam	24.1	8.5	18.6	13.7	Silt soil	Y
Mwamgongo (Tanzania)	(-4.62742, 29.65162)	676m	Stream	25.9	6.8	28.1	13.7	Silt soil	Y

Table 9: Table of sites visited where *B. pfeifferi* snails were found in East Africa's River systems.

B. pfeifferi snails were found at 23/31 sites where *Biomphalaria* snails were found (Figure 36). Streams were the habitat type preferred by *B. pfeifferi* snails; (17/23 sites) where *B. pfeifferi* were found were streams, revealing the preference of *B. pfeifferi* for streams. The other habitat types where *B. pfeifferi* snails were found were irrigation schemes, rivers, and dams.

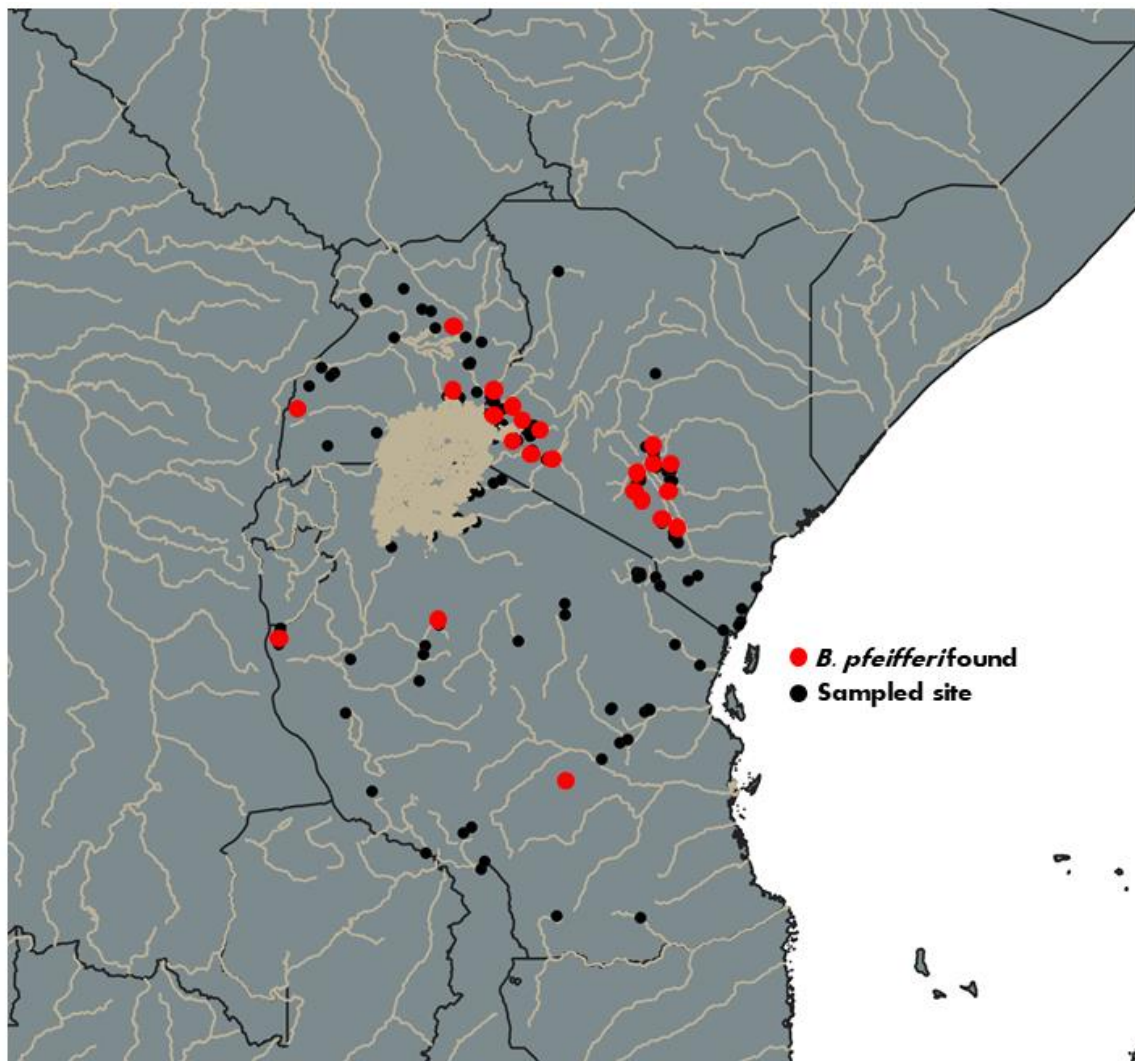


Figure 36: A map of East Africa showing sites where *B. pfeifferi* snails were found.

Biomphalaria snails belonging to the 'Nilotic Species Complex' were found at 9 sites: Kabere (Uganda), Masindi Port (Uganda), Mabogini (Tanzania), Kibimba (Uganda), Lubiri (Uganda), Babati (Tanzania), Muzizi (Uganda), Ntoroko (Uganda) and Kole (Uganda) (Table 10 and Figure 37).

Name of site	Geographic Coordinates	Altitude	Type of habitat	Water Temp (°C)	pH	Water depth (cm)	Water velocity (cm/s)	Soil Type	Veg (Y/N)
Kabere (Uganda)	(0.41284, 33.49619)	1156m	Stream	21.3	7.2	19.2	7.1	Silt soil	Y
Kibimba (Uganda)	(0.52956, 33.85815)	1080m	Irrigation scheme	24.7	7.3	14.6	11.2	Silt soil	Y
Kole (Uganda)	(2.302313, 32.68811)	1056m	River	21.3	6.3	19.2	6.4	Silt soil	Y
Mabogini (Tanzania)	(-3.40061, 37.36256)	684m	Irrigation scheme	24.3	8.8	23.6	12.3	Silt soil	Y
Masindi (Uganda)	(1.69541, 32.09238)	1034m	River	19.2	6.7	26.4	7.4	Silt soil	Y
Muzizi (Uganda)	(0.87095, 30.72997)	1178m	River	21.1	9.2	21.5	9.6	Silt soil	Y
Ntoroko (Uganda)	(1.05375, 30.53696)	631m	River	20.8	8.4	28.4	10.4	Silt soil	Y
Lubiri (Uganda)	(2.45857, 31.49975)	632m	River	22.1	7.3	19.7	12.5	Silt soil	Y
Babati (Tanzania)	(-4.227588, 35.744211)	979m	River	24.5	8.1	27.2	8.3	Silt soil	Y

Table 9: Table of sites visited where where *Biomphalaria* snails belonging to the Nilotic Species complex were found.

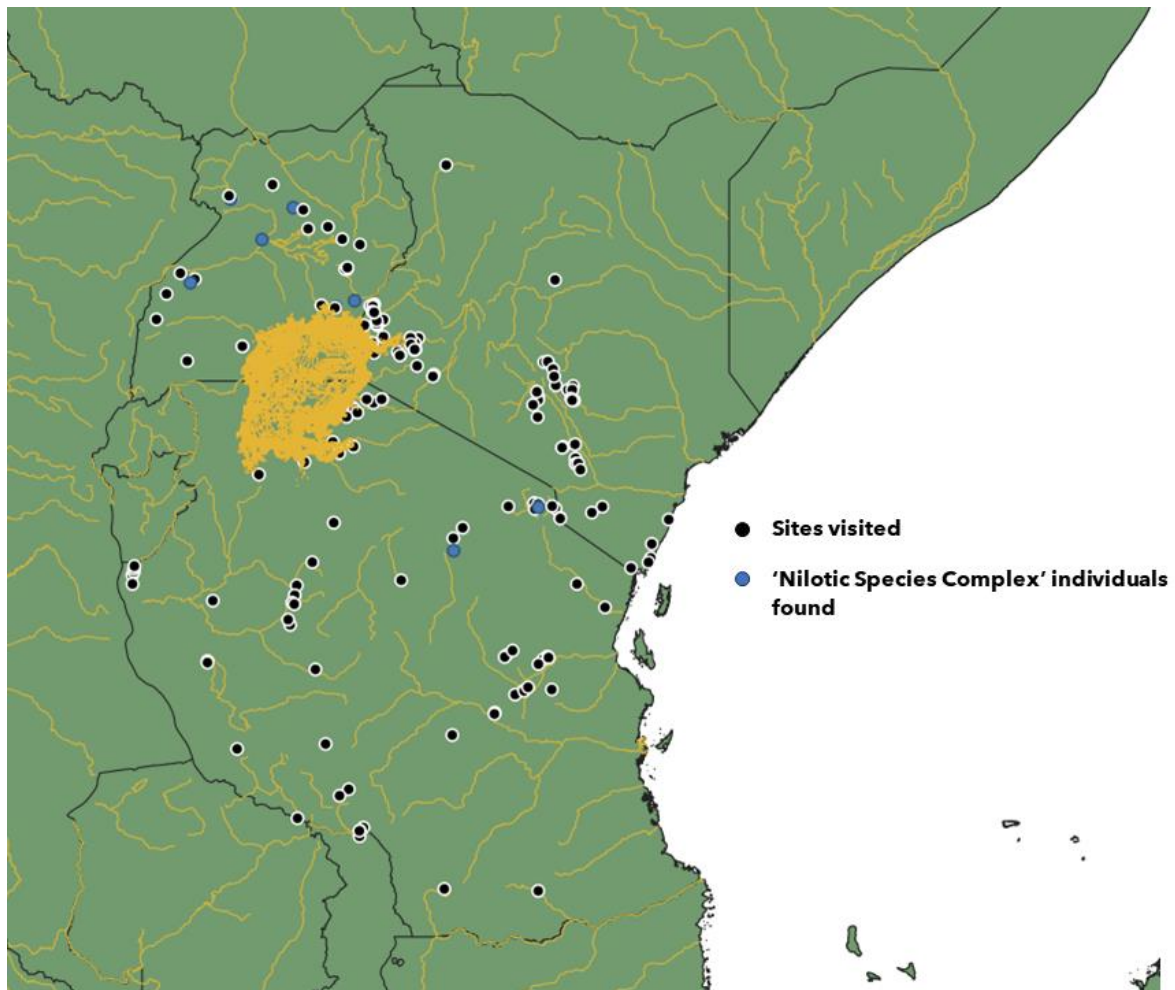


Figure 37: A map of East Africa showing sites where *Biomphalaria* snails belonging to the Nilotic Species complex were found.

4.2.3 Ecological and physicochemical parameters

Geographical coordinates, ecological parameters and physicochemical parameters associated with snail abundance were recorded. Data on vegetation availability, type of vegetation (algae), water temperature, soil type, presence of competitor snails, pH, water depth and water velocity were recorded for each site (all the 172 sites sampled). Water temperature at the sites ranged from 19.1°C – 27.4°C and the median (interquartile range) water temperature was 23.6°C (21.18°C–25.23°C). The water-depth of the sites ranged from 19.3cm – 41.3cm and the median (interquartile range) water depth was 27.45 cm (23.1cm – 32.4cm). Based on negative binomial regression in glmmTMB, only temperature and water depth had a statistically significant positive relationship with the abundance of *Biomphalaria* snails (p-values of 0.00184 and 0.0187 respectively). The other variables (vegetation, algae, pH, soil type and presence of competitor snails) did not show any statistically significant relationship with the abundance of *Biomphalaria* snails (Table 11).

Variables	Estimate	Std. Error	Confidence Interval	p-value
Intercept	-7.11562	3.93026	-14.81878984 - 0.5875451	0.07022
Vegetation	1.82671	1.48377	-1.08143734 - 4.7348507	0.21828
Algae	0.18526	1.10139	-1.97341919- 2.3439365	0.86642
Temp	0.2508	0.08051	0.09300019 - 0.4085942	0.00184 **
pH	0.33459	0.43332	-0.51471196- 1.1838902	0.44003
Water-depth	-0.07316	0.03113	-1.341682e-01 -- 1.215556e-02	0.0187 *
Soil type	-0.51384	0.31513	- 1.131483e+00 - 1.038082e-01	0.1030
Competitor snails	-23.64088	9561.62528	-1.876408e+04 - 1.871680e+04	0.9980
Water velocity	-0.01207	0.02130	-5.381777e-02- 2.967123e-02	0.5708

*Statistical significance at $p < 0.05$

Table 11: The correlation between snail abundance with ecological and physicochemical parameters courtesy of glmmTMB in R for the 172 sites.

4.3 DISCUSSION

This study presents an extensive survey of *Biomphalaria* snails in East Africa's River system with sampling undertaken from 172 sites across Kenya, Uganda and Tanzania. It was established that *Biomphalaria* snails are widely distributed across East Africa, outside Lake Victoria and Lake Albert with *B. pfeifferi* being the *Biomphalaria* species most commonly found (at 23/31 sites), indicating that it is the most distributed *Biomphalaria* species in East Africa's River systems.

This study also proved that *Biomphalaria* snails in East Africa's River systems have a preference for streams as a habitat (61.3% of all the sites where the snails were found were streams), followed by rivers (22.58%), irrigation schemes (9.68%) and dams (6.45%). In addition, streams were the habitat type preferred by *B. pfeifferi* snails (17/23 sites where *B. pfeifferi* were found were streams); asserting the preference of *B. pfeifferi* for streams. On

the other hand, rivers were the habitat type preferred by members of the 'Nilotic Species Complex' (6/8 of sites where 'Nilotic Species Complex' snails were found were rivers). This suggests that streams are the most ideal aquatic habitats for *Biomphalaria* snails, outside the lake environment.

B. pfeifferi snails in Kenya were found around the Lake Victoria basin (Kisumu County, Busia County, Siaya County, Kisii County, Homa Bay County and Nyamira County); at Mwea irrigation scheme, Machakos County, Kitui County and Makueni County. *B. pfeifferi* snails in Uganda were found around the Lake Victoria basin (Jinja District), in the Albertine region (Ntoroko district) and Soroti District. *B. pfeifferi* snails in Tanzania were found in the Iringa region, Tabora region and Kigoma region. In Uganda, 'Nilotic Species Complex' individuals were found around the Lake Victoria basin (Bugiri and Mayuge districts), in Masindi District, Ntoroko District, in Kole District, Kyenjojo District and Pakwach District. In Tanzania, individuals belonging to the 'Nilotic Species Complex' were found in the Kilimanjaro region and Manyara region. Transmission of schistosomiasis is not possible in areas where there is absence of snail intermediate hosts and therefore, understanding the distribution of the snails has a role to play in prevention and control of the disease. This knowledge on the distribution of *Biomphalaria* snails in East Africa's River systems can support surveillance responses in terms of detecting new transmission foci and potential schistosomiasis hotspots.

Ecological and physicochemical parameters have an influence on the distribution of *Biomphalaria* snails. Temperature was established to be a crucial factor as far as geographical distribution of the snails is concerned with water temperature established to be the most significant parameter that influences the distribution of the snails (p -value= 0.00184). Temperature as a climatic factor is very important for the survival of *Biomphalaria* snails (Yang *et al.*, 2018). Sites where no snails were found had temperatures above 30°C and others had temperatures below 15°C. Sites where less than 20 snails were found had a temperature range of between 19.1°C to 21.3°C. On the other hand, sites where more than 20 snails were found had a temperature range of between 23.2°C to 26.1°C. This suggests that temperature has a role to play in the abundance of *Biomphalaria* snails in aquatic habitats. According to Yang *et al.* (2018), ideal temperature is important for the fecundity of snail intermediate hosts. Sturrock (1965) states that *Biomphalaria* snails are active at temperatures of between 18°C and 32°C while reproduction and survival of the snails is optimum at a temperature range of between 20°C and 26°C. Optimum temperature is also important as far as the production of cercariae is concerned (Yang *et al.*, 2018). Previous studies suggest that *Biomphalaria* snails are not as temperature tolerant as *Bulinus* snails and high temperatures discourage their habitation (Sturrock, 1965). Our findings reveal that sites that had temperatures of above 27°C had low snail densities compared to sites that had temperatures of around 25°C. This explains why *Bulinus* snails have previously been found in arid and semi-arid areas whereas *Biomphalaria* snails are rarely found in such environmental conditions (Sturrock, 1965). Our findings corroborate with a study by Ofulla *et al.* (2013) which avers that *Biomphalaria* snails are less tolerant of temperatures above 27°C. Habib *et al.* (2021) asserts that temperature is an important factor for *Biomphalaria* snails' reproduction and survival and that temperature changes may alter the breeding, survival, and geographical distribution of the snails. A positive correlation between water temperature and *B. sudanica* has also been reported previously

(Kazibwe *et al.*, 2006). Temperatures higher than optimal values negatively interfere with egg production and development of reproductive organs (Habib *et al.*, 2021).

Water-depth was also established to be of statistical significance in relation to *Biomphalaria* snail abundance (p-value=0.0187). Snail abundance decreased with increase in water depth. High snail densities were found in Kakulutuine stream, a shallow stream less than 0.3 m deep and the high snail density could be attributed to the shallowness of the stream. A study by Magendantz (1972) asserts that water depth is an important factor as far as distribution of snail intermediate hosts is concerned and that there is a correlation between shallowness of a water body and the abundance of the intermediate hosts. In addition, Ofulla *et al.*, (2013) showed a negative correlation between snail density and water depth in aquatic habitats of the Lake Victoria basin. It is worth pointing out that water is an important factor for the survival of snails but too much of it decimates snail populations (Manyangadze *et al.*, 2021).

Presence of vegetation in aquatic habitats has been reported to play a role in the distribution of freshwater snails (Lodge *et al.*, 1987). Although we did not find statistical significance between vegetation and *Biomphalaria* abundance for all the 172 sites (p-value=0.21828), previous studies have reported that aquatic plants play an important role in the distribution and habitation of snails because they provide the necessary conditions for feeding, oviposition, breeding as well as shelter (Kloos *et al.*, 2004).

Soil type has been shown to have an influence on *Biomphalaria* abundance (Ofulla *et al.*, 2013). While we did not find statistical significance between soil type and *Biomphalaria* abundance (p-value=0.21828) for all 172 sites, we observed that muddy soils tended to promote the habitation of the snails. Muddy soils have been noted to be important as far as survival of *Biomphalaria* snails is concerned because they protect snails from desiccation during hot and dry seasons (Lydig, 2009). Sandy soil is not conducive for the habitation of *Biomphalaria* snails and low snail abundance was found in water bodies rich in sandy soil. An example is Tulimuimbu stream, in Eastern province, Kenya that had lower snail abundance (n=7) and was characterised by sandy soil. Lydig (2009) posits that rich black cotton soils that are rich in organic matter are favourable for the habitation of *Biomphalaria* snails. According to Ofulla *et al.* (2013), the substratum in any given habitat has a role to play in the availability of snails and clay soil and silt constitute very important substrata favourable for the survival of *Biomphalaria* snails. Therefore, absence of *Biomphalaria* snails in a given location could be due to the lack of the right substrata.

Water velocity has been established to have an influence on the distribution and abundance of freshwater snails (Rabone *et al.*, 2019; El-Deeb *et al.*, 2017; Ntonifor & Ajayi, 2007). In spite of the fact that we did not find statistical significance between water velocity and *Biomphalaria* abundance (p-value= 0.5708), it has been reported previously that *Biomphalaria* snails are not found in sites with water velocities greater than 30cm/s (Appleton, 1978). We observed that water velocity higher than 30cm/s negatively correlated with *Biomphalaria* snail abundance. Water velocities higher than 30cm/s are a limiting factor as far as snail abundance is concerned. According to Appleton (1978), water velocities have a tendency to sweep *Biomphalaria* snails from one location to another,

mostly during the rainy season. Floods are a bottleneck that has been confirmed to have a direct impact on the availability and distribution of *Biomphalaria* snails (Habib *et al.*, 2021).

Competitor snails of *Biomphalaria* snails are regarded as biotic factors that affect the distribution of the intermediate hosts (Pointier *et al.*, 1993). While we did not find statistical significance between the presence of *Melanooides tuberculata* (a competitor snail) and the abundance of *Biomphalaria* snails (p-value=0.9980), previous studies affirm that *M. tuberculata*, a highly invasive snail species is very effective in displacing *Biomphalaria* snails from their habitats (Gashaw *et al.*, 2008 and Pointier, 1993). *Biomphalaria* snails were absent in most of the sites where we found *M. tuberculata* in our study except at Ntoroko (Western Uganda). Competitor snails of *Biomphalaria* snails in East Africa would therefore seem to have an effect on the distribution of *Biomphalaria* snails in the region albeit not significant in our glmmTB analyses. Competitor snails have been used in schistosomiasis control by eliminating the snail intermediate host thus interrupting the transmission of *Schistosoma* parasites. Natural enemies of *Biomphalaria* snails such as *Marisa* snail that competes with *Biomphalaria* snails for food and also eats *Biomphalaria* eggs has been used to control *Biomphalaria* snail populations. *Marisa* species are widely distributed in South America, Africa, Central America and the Caribbean. Introduction of *Marisa* snails in habitats occupied by *Biomphalaria* snails has played a major role in decimating *Biomphalaria* populations thus contributing towards schistosomiasis control (Cedeno-Leon & Thomas, 1982).

4.4 CONCLUSION

This study showed that *Biomphalaria* snails are widely distributed in East Africa's River systems with *B. pfeifferi* being the most widely distributed *Biomphalaria* species. The geographical distribution of *Biomphalaria* snails is influenced by ecological and physicochemical factors, with temperature and water depth being the most important factors that influence the distribution of the snails. This study provides information on the distribution of *Biomphalaria* snails in East Africa's River systems and contributes towards the body of knowledge on schistosomiasis and snail intermediate hosts. Although snail sampling was conducted extensively in East Africa, further sampling should be conducted in future because of the seasonality of the water bodies that are inhabited by the snails and the ravages caused by floods and droughts to those very habitats.

CHAPTER FIVE: MOLECULAR DETECTION OF *S. MANSONI* INFECTIONS IN *BIOMPHALARIA* SNAILS FROM EAST AFRICA'S RIVER SYSTEMS

ABSTRACT

Schistosomiasis is a major public health problem in East Africa, with an estimate of 40 million people infected with the disease. In this study, we used molecular techniques to determine the incidence of schistosome infection in *Biomphalaria* snails found in East Africa's River systems. The essence of this study was to create a basis for which a schistosomiasis surveillance system could be established in East Africa that exclusively deals with molecular detection of schistosome infection in snail intermediate hosts. *S. mansoni* infections were detected in snails from 11 out of the total 31 *Biomphalaria* snail populations. Kenya had the highest number of *S. mansoni* infected *Biomphalaria* populations (8 populations), followed by Uganda at 2 populations and lastly Tanzania at 1 population. At the species level, 11 out of the 11 infected *Biomphalaria* sites were *B. pfeifferi* populations with no populations that belong to the 'Nilotic Species Complex' being found infected. Infection rates in Kenyan sites ranged from 10% to 40%; infection rates in Ugandan sites ranged from 20% to 60% and the infection rate in the singular Tanzanian site was 10%. The results in this study suggest that the detection of schistosome infection in snail intermediate hosts can serve as a time saving surveillance approach and that *B. pfeifferi* is arguably the most important snail intermediate host in the transmission of intestinal human schistosomiasis in East Africa, outside Lake Victoria and Lake Albert.

5.1 INTRODUCTION

Schistosomiasis is a neglected tropical disease that is caused by flatworms of the Genus *Schistosoma*. Approximately 85% cases of schistosomiasis occur in sub-Saharan Africa and 7% of cases occur in South America (Colley *et al.*, 2014). In East Africa, it is estimated that about 40 million people are infected with schistosomiasis and about 140 million people are at risk of infection (Aula *et al.*, 2021). There are localities in East Africa where the prevalence of schistosomiasis has been reported to be high as 90% in humans and localities where the prevalence of schistosomiasis has been reported to be as low as 2% in humans (Aula *et al.*, 2021).

The main species that cause human schistosomiasis are *Schistosoma mansoni* (a causative agent of human intestinal schistosomiasis) and *Schistosoma haematobium* (a causative agent of human urogenital schistosomiasis). About 1 billion people are currently affected by schistosomiasis, 250 million people are infected and about 200,000 deaths that occur annually are attributed to the disease (Hlengiwe *et al.*, 2018). The life cycle of *Schistosoma* parasites involves a definitive host and a snail intermediate host, and the distribution of the parasites is dependent on the intermediate host's habitat range. Snails of the genus *Biomphalaria* serve as intermediate hosts for *S. mansoni* and snails of the genus *Bulinus* serve as the intermediate hosts of *S. haematobium* (Colley *et al.*, 2014).

Schistosomiasis is of major economic and public health relevance because of the disabilities, morbidity and mortality associated with the disease. Schistosome infection has debilitating effects on infected individuals, rendering them unproductive due to physical disability and malaise that result from the infection (Gryseels, 2012). It is estimated that Schistosomiasis causes about 3.5 million disability adjusted life years (Tchuem *et al.*, 2017). Disability adjusted life years (DALYs) refer to the total number of wasted years due to the inability to work because of malaise and physical disability that stem from schistosome infection (Gryseels, 2012).

There are several studies that have studied the prevalence of intestinal schistosomiasis in East Africa. It has been shown that the prevalence of intestinal schistosomiasis in Uganda consistently exceeded 50% in areas located 5 km from lakes Albert, Victoria and Kyoga (Brooker *et al.*, 2006). It has also been revealed that the predicted risk of intestinal schistosomiasis infection in North-Western Tanzania is higher than 50% (Brooker *et al.*, 2006). Schistosomiasis has been shown to be endemic in several locations in Kenya, more so in Western Kenya where in some instances, prevalence has been reported to be as high as 100% (Matete *et al.* 2015; Odiere *et al.* 2012).

School aged children, teenagers, women, fishermen and sand harvesters constitute demographic groups that are most susceptible to being infected by schistosomes because they spend a large amount of their time in waters that are infested with schistosome infected snails. Swimming, washing clothes and utensils, playing in water, fishing and sand harvesting are some of the human activities that make human beings more susceptible to schistosome infection (Colley *et al.*, 2014).



Figure 38: School aged children playing, swimming, and washing clothes in Dago stream. Dago stream (-6.8934, 37.07775) is located a few kilometres from Kilosa town, Morogoro region, Tanzania.

There is a need for rapid detection of schistosome infection to promptly respond and intervene on existing and emerging cases. Molecular detection of schistosome infections in snail intermediate hosts offers a better surveillance alternative, because of its rapidity compared to traditional surveillance methods of detecting schistosome eggs in faeces and urine that have a low sensitivity and are slow in process or cercarial shedding in snails (Rollinson *et al.*, 2009).

Traditionally, schistosome infection detection in snail intermediate hosts has involved cercarial shedding (Weerakoon *et al.*, 2015). Cercarial shedding involves exposing snails suspected to be infected with schistosomes to sunlight under field conditions, allowing emergence of cercaria from the snails. Infection rate in naturally infected *B. pfeifferi* snails found in Gombe ecosystem of Western Tanzania was found to be 12%, on cercarial shedding (Bakuza *et al.*, 2017). Sturrock *et al.* (1979) revealed infection rates ranging from 3.1% to 34% in naturally infected *B. pfeifferi* snails found in Kenya, by cercarial shedding. Thirdly, naturally infected *Biomphalaria* snails from informal settlement schemes in Kisumu (Western Kenya) were reported to have an infection rate of between 1.6% to 1.7% as revealed by cercarial shedding (Opisa *et al.*, 2011). However, cercarial shedding techniques have serious limitations. They can only identify patent infection, meaning that snails with prepatent infection are treated as uninfected snails (Bakuza *et al.*, 2013). Cercarial shedding techniques are also very time insensitive (Nwoko *et al.*, 2021).

Polymerase Chain Reactions techniques have been developed to detect schistosome infection in snail intermediate hosts, courtesy of the advancement in molecular biology (Hamburger *et al.* 1998; Sandoval *et al.* 2016; Lu *et al.* 2016). PCR techniques show a high level of schistosome infection detection by detecting prepatent stages of the parasites in the snail intermediate hosts compared to the level of infection demonstrated by cercarial shedding (Joof *et al.*, 200). They also provide a rapid alternative to traditional surveillance methods. There are various studies that have detected schistosome infection in snail intermediate hosts using molecular methods. A study by Farghaly *et al.* (2016) detected

prepatent *S. mansoni* infection in *B. alexandrina* by targeting a specific gene (SMALDO gene) and the results were characterised by high sensitivity and specificity. A nested Polymerase Chain Reaction (PCR) protocol developed by Hanelt *et al.* (1997), detected *S. mansoni* infection in *B. alexandrina* from Egypt with a 92% detection rate. A study by Caldeira *et al.* (2017), detected *S. mansoni* DNA in *B. glabrata*, *B. straminea* and *B. tenagophila* with high sensitivity and high specificity using low stringency- polymerase chain reaction (LS-PCR) and loop mediated isothermal amplification (LAMP). Infection rate in *B. pfeifferi* snails found in Gombe ecosystem of Western Tanzania was found to be 47% when a PCR technique was used, from 12% cercarial shedding results (Bakuza *et al.*, 2017). A study by Casotti *et al.* (2020), detected *S. mansoni* infection in *B. glabrata* from Brazil using the Sandoval PCR technique. Another recent study revealed an infection rate of 35.4% in naturally infected *Biomphalaria* snails from Ijinga island, north-western Tanzania using a PCR technique (Fuss *et al.*, 2020).

In East Africa, *B. pfeifferi* has been shown to be more compatible with *S. mansoni* when compared to the compatibility that *S. mansoni* shows with other *Biomphalaria* snails (Mutuku *et al.* 2017; Lu *et al.* 2016). On the other hand, the compatibility of *S. mansoni* with *B. choanomphala* seems to be higher than the compatibility of *S. mansoni* with *B. sudanica*. In a study by Trienekens *et al.* (2022), 66.67% of the *Biomphalaria* snails that were shown to be infected with *S. mansoni* were *B. choanomphala* and 33.33% were *B. sudanica*. Mutuku *et al.* (2021) asserts that *B. choanomphala* is more compatible with *S. mansoni* than *B. sudanica*. In Ugandan Lake Victoria, *B. choanomphala* is regarded as the most important transmitter of *S. mansoni* because more naturally *S. mansoni* infected *B. choanomphala* populations have been found than naturally *S. mansoni* infected *B. sudanica* populations (Standley *et al.*, 2011). Magendantz (1972) showed higher *S. mansoni* infection rates in *B. choanomphala* than *B. sudanica* in *Biomphalaria* snails from Tanzania's Lake Victoria.

The primary objective of this study was to detect *S. mansoni* infection from *Biomphalaria* snails collected in the field. Conducting malacological surveys periodically in a region that is endemic or suspected to harbour schistosome infection followed by screening of the snail intermediate hosts for schistosome infection using molecular techniques can be a noble surveillance approach in revealing hotspots and areas with emerging cases of schistosomiasis.

5.2 RESULTS

S. mansoni specific primers were used to detect schistosome infection in *Biomphalaria* snails. Schistosome infection was detected at 11 out of the total 31 sites where *Biomphalaria* populations were found with Kenya having the highest number of *S. mansoni* infected *Biomphalaria* populations (8 populations), followed by Uganda at 2 populations and lastly Tanzania at 1 population) (Table 12).

Name of Site	Infection rate
Ambururu (Kenya)	1/10
Asawo (Kenya)	4/10
Babati (Tanzania)	0/10
Bwawani (Tanzania)	0/10
Cheptuyet (Kenya)	0/10
Itua (Kenya)	1/10
Ivuta (Kenya)	0/10
Kabere (Uganda)	0/1
Kakulutuine (Kenya)	2/10
Kalundu (Kenya)	0/10
Kiangangi (Kenya)	0/10
Kibimba (Uganda)	0/10
Kodongo (Kenya)	2/10
Kodumo (Kenya)	2/10
Kole (Uganda)	0/3
Kwase (Kenya)	0/2
Lubiri (Uganda)	0/10
Mabogini (Tanzania)	0/10
Masindi (Uganda)	0/10
Matingani (Kenya)	1/10

Mwamgongo (Tanzania)	1/10
Mukou (Kenya)	0/10
Musilili (Kenya)	0/10
Muzizi (Uganda)	0/7
Ntoroko (Uganda)	3/5
Onsando (Kenya)	0/10
Opiyai (Uganda)	2/10
Rakite (Kenya)	0/5
Tulimuimbu (Kenya)	1/7
Utwigu (Tanzania)	0/10
Walukuba (Uganda)	0/10

Table 12: Table of sites where *Biomphalaria* snails were found with their respective *S. mansoni* infection rates.

In Kenya, we detected *S. mansoni* infection at Ambururu (1/10 snails, 10%), Asawo (4/10, 40%), Itua (1/10, 10%), Kakulutuine (2/10, 20%), Kodongo (2/10, 20%), Kodumo (2/10, 20%), Matingani (1/10, 10%), Mwamgongo (1/10, 10%), Ntoroko (3/5, 60%), Opiyai (2/10, 20%) and Tulimuimbu (1/7, 14%). In Uganda, *S. mansoni* infection was detected at Ntoroko (3/5 snails, 60%) and Opiyai (2/10, 20%). In Tanzania, *S. mansoni* infection was detected at Mwamgongo (1/10 snails, 10%) (Table 12). We observed low to moderate incidences of *S. mansoni* infection with an overall infection rate in infected sites of 21.27%, ranging from 10% to 60%. In Kenya, *S. mansoni* infected snails were found in the Lake Victoria basin and the former Eastern Province with an average infection rate of 18%. In Uganda, infected snails were found in the Ntoroko District and Soroti District with an average infection rate of 40%. In Tanzania, infected snails were found in the Kigoma region with an infection rate of 10%. Of the 11 populations that were found infected with *S. mansoni*, all of them were *B. pfeifferi* populations; none were 'Nilotic Species Complex' populations.

Kenya had the highest number of *S. mansoni* infected *Biomphalaria* populations at 72.7% of sites, followed by Uganda at 18.2% of sites and Tanzania at 9.1% of sites (Figure 39).

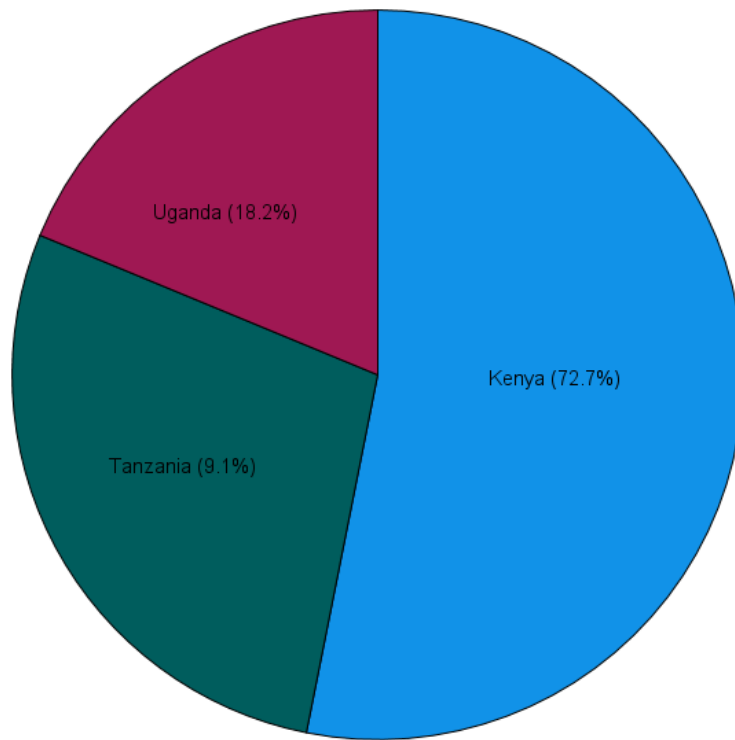


Figure 39: Pie chart showing per country proportion of *S. mansoni* infected sites in East Africa's River systems.

Chi-square tests (Fisher's Exact Test in particular) affirmed that Kenya had the highest number of *S. mansoni* infected sites. On ascertaining the statistical significance of Kenya having a higher number of *S. mansoni* infected sites than Uganda, the p-value was significant (p-value=0.001) (Table 13). On ascertaining the statistical significance of Kenya having a higher number of infected sites than Tanzania, the p-value was also significant (p-value=0.039) (Table 14).

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2- sided)	Exact Sig. (1- sided)	Point Probability
Pearson Chi-Square	18.471 ^a	1	<.001	<.001	<.001	
Continuity Correction ^b	17.060	1	<.001			
Likelihood Ratio	18.835	1	<.001	<.001	<.001	
Fisher's Exact Test				<.001	<.001	
Linear-by-Linear Association	18.366 ^c	1	<.001	<.001	<.001	.000
N of Valid Cases	177					

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 23.16.

b. Computed only for a 2x2 table

c. The standardized statistic is -4.286.

Table 13: Chi-square tests testing the statistical significance of Kenya having a higher number of *S. mansoni* infected sites when compared to Uganda (p-value=0.001)

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2- sided)	Exact Sig. (1- sided)	Point Probability
Pearson Chi-Square	4.090 ^a	1	.043	.051	.039	
Continuity Correction ^b	3.155	1	.076			
Likelihood Ratio	3.990	1	.046	.051	.039	
Fisher's Exact Test				.051	.039	
Linear-by-Linear Association	4.062 ^c	1	.044	.051	.039	.027
N of Valid Cases	147					

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 7.89.

b. Computed only for a 2x2 table

c. The standardized statistic is -2.016.

Table 14: Chi-square tests testing the statistical significance of Kenya having a higher number of *S. mansoni* infected sites when compared to Tanzania (p-value =0.039).

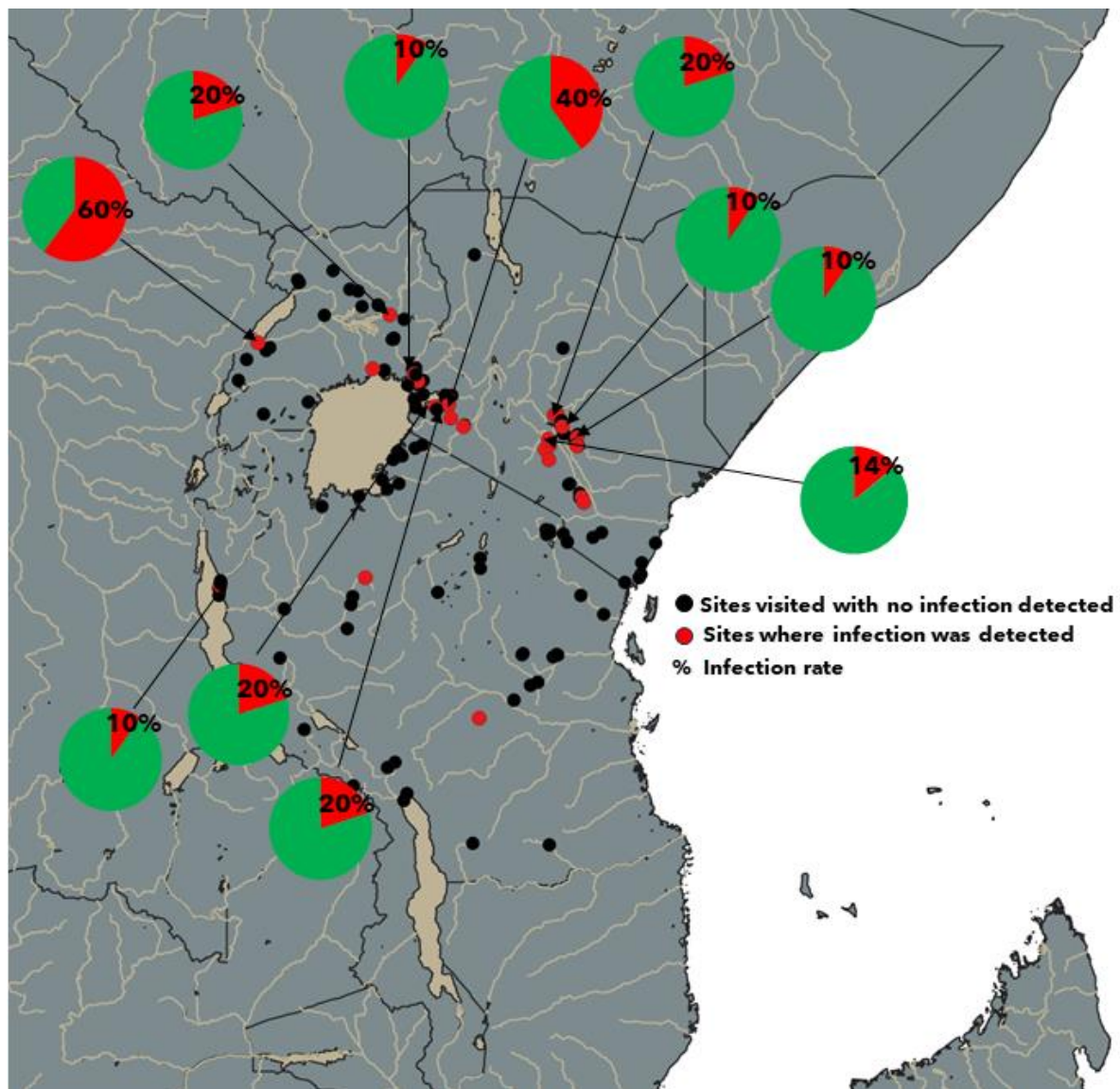


Figure 40: Map of East Africa showing *S. mansoni* infection rates in *Biomphalaria* snails from East Africa's River systems. *B. pfeifferi* was the only *Biomphalaria* species infected with *S. mansoni*.

The schistosome infection rate in *B. pfeifferi* when compared to the infection rate in 'Nilotic Species Complex' was found to be statistically significant (Table 15).

Chi-Square Tests						
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)	Point Probability
Pearson Chi-Square	60.131 ^a	39	.016	.006		
Likelihood Ratio	42.067	39	.340	.007		
Fisher-Freeman-Halton Exact Test	47.530			.007		
Linear-by-Linear Association	14.742 ^b	1	<.001	<.001	<.001	.000
N of Valid Cases	25					

a. 56 cells (100.0%) have expected count less than 5. The minimum expected count is .08.

b. The standardized statistic is -3.840.

Table 15: Comparing the infection rate in *B. pfeifferi* with the 'Nilotic Species Complex' to ascertain whether the infection rate in *B. pfeifferi* is statistically significant.

5.3 DISCUSSION

Monitoring and surveillance of potential schistosomiasis transmission sites is important because this can help reduce morbidity and mortality cases related to the disease before such cases escalate. Detection of *S. mansoni* infection in *Biomphalaria* snails in East Africa's River systems is important because in doing so; we can identify new transmission foci as well as persistent transmission hotspots.

Molecular detection of schistosome infection in snail intermediate hosts is also less laborious and less strenuous compared to detecting schistosome infection in human subjects. Traditional processing of faecal and urine for parasitological diagnostic purposes can also be cumbersome and unpleasant. Therefore, molecular detection of schistosome infection in snail intermediate hosts offers a better alternative to ascertaining prevalence, incidences, and intensity of schistosome infection. In addition, there is a need to set up better monitoring and surveillance systems in regions where schistosomiasis is prevalent or likely to be prevalent.

S. mansoni infection is more prevalent in *Biomphalaria* snails from Kenya than in *Biomphalaria* snails from the other East African countries of Uganda and Tanzania and this was proven to be statistically significant (p-value=0.001; p-value =0.039). *B. pfeifferi* seems to be the *Biomphalaria* species in East Africa's River systems that is most susceptible to *S. mansoni* infection; 100 % of infected sites were *B. pfeifferi* sites.

Biotic and abiotic factors are the likely contributors to *B. pfeifferi* snails being more susceptible to *S. mansoni* infection when compared to the other *Biomphalaria* snails in East Africa's River systems. A study by Mutuku *et al.* (2014) investigated the compatibility of *S. mansoni* with *B. pfeifferi* in Kenya and stated that persistent self-fertilisation and geographical isolation (two factors that increase genetic homozygosity) of *B. pfeifferi* populations could be major factors that contribute towards heightened compatibility of *S. mansoni* with *B. pfeifferi* populations. Several studies have associated genetic heterozygosity of hosts with resistance to some infectious diseases (Penn *et al.*, 2002; Bu *et al.*, 2002 & Campbell *et al.*, 2010). The high infection rate in *B. pfeifferi* populations

supports the hypothesis that lack of allelic richness in *Biomphalaria* populations increases success rates of parasitic infections.

On the other hand, it has been reported that *B. choanomphala* and *B. sudanica* are relatively less compatible with *S. mansoni* (Frandsen, 1979). In the study by Frandsen (1979), different populations of *B. pfeifferi*, *B. sudanica*, *B. choanomphala*, *B. glabrata* and *B. alexandrina* were exposed to different strains of *S. mansoni*; the infection rate in *B. pfeifferi* snails ranged from 50%- 90% whereas the infection rate in *B. choanomphala* ranged from 24% to 38% and the infection rate in *B. sudanica* ranged from 2.8% to 33%. In a study by Lu *et al.* (2016), *S. mansoni* was more compatible with *B. pfeifferi* than with *B. sudanica*.

We recommend follow up in the geographical areas where *S. mansoni* infection was high in the *Biomphalaria* snail populations by conducting diagnostic tests for the presence of *S. mansoni* infection in human subjects in areas and communities close to habitats of the infected *Biomphalaria* snails. With the follow up, we will be able to establish the prevalence of *S. mansoni* infection in human populations in the respective areas and communities and subsequently initiate relevant prevention and control strategies.

5.4 CONCLUSION

In conclusion, we ascertained that the molecular detection of schistosome infection in snail intermediate hosts can form a basis for the development of a better monitoring and surveillance strategy geared towards prevention and control of schistosomiasis. The use of molecular methods to detect schistosome infection has been proven to be a time-saving and rapid diagnostic approach. After the detection of schistosome infection in snail intermediate hosts, follow up can then be done to detect schistosome infection in human populations that live close to the snail populations' habitats. In this study we used *S. mansoni* specific primers designed by Lu *et al.* (2016) amplifying the ND5 gene and Sandoval primers amplifying the 28S rDNA region to detect *S. mansoni* infections in *Biomphalaria* snails' DNA samples. *S. mansoni* infections were detected in snails from 11 out of the total 31 *Biomphalaria* snail populations with Kenya having the highest number of *S. mansoni* infected *Biomphalaria* populations (8 populations), followed by Uganda at 2 populations and lastly Tanzania at 1 population. All the *Biomphalaria* populations that were found infected with *S. mansoni* were *B. pfeifferi*, no 'Nilotic Species Complex' population was found infected. The results herein agree with previous studies that suggest that *B. pfeifferi* is more compatible with *S. mansoni* when compared to the compatibility that *S. mansoni* shows with other *Biomphalaria* snails. Chi-square tests testing the statistical significance of Kenya having a higher number of *S. mansoni* infected sites when compared to Uganda and Tanzania confirmed that the significance was indeed statistically significant. The schistosome infection rate in *B. pfeifferi* samples when compared to the infection rate in 'Nilotic Species Complex' samples was also found to be statistically significant. Several studies have associated genetic heterozygosity of hosts with resistance to some infectious diseases. The high infection rate in *B. pfeifferi* populations supports the hypothesis that lack of allelic richness in *Biomphalaria* populations increases success rates of parasitic infections. It has also been reported that *B. choanomphala* and *B. sudanica* are relatively less compatible with *S. mansoni*; this agrees with the current study where there was no schistosome infection detection in any of the 'Nilotic Species Complex' populations. Given

that all *Biomphalaria* snails that were infected with *S. mansoni* were *B. pfeifferi* snails and no member of the 'Nilotic Species Complex' was found infected, it implies that *B. pfeifferi* is more compatible with *S. mansoni* when compared to the other East African *Biomphalaria* species and arguably the most important *Biomphalaria* species in the transmission of intestinal schistosomiasis in East Africa, outside Lake Victoria and Lake Albert.

CHAPTER SIX: POPULATION GENETICS OF PRE-PATENT AND SUB-PATENT *S. MANSONI* POPULATIONS

ABSTRACT

The population genetic structure of *Schistosoma* parasite populations has a role to play in their survival, infectivity, and resistance to antischistosomal drugs. It is also paramount to understand the selection pressures that potentially shape the population genetic structure of *S. mansoni* populations. In this study, we investigated the phylogenetic relationship of several *Schistosoma* species using the ND5 mitochondrial gene and investigated the population genetic structure of prepatent and sub-patent *S. mansoni* populations by analysing ND5 mitochondrial gene sequences from NCBI and those generated from our fieldwork samples. Genetic variation within our fieldwork *S. mansoni* populations was 53.34% and genetic differentiation among the populations 46.66% with an F_{ST} value of 0.46660 suggestive of moderate gene flow. Probable causes of moderate levels of genetic variation are random mating, moderate to high levels of geneflow and the ability of *Schistosoma* parasites to quickly adapt to new challenges and environments. Results from this study could be interpreted as suggesting that the population genetic structure of *S. mansoni* parasites can potentially be a hindrance to the success of preventive chemotherapy efforts in East Africa because of the innate ability of the parasite to handle selection pressures courtesy of heightening the genetic diversity of its populations which makes it resistant to different selection pressures.

6.1 INTRODUCTION

Schistosoma mansoni is one of the most abundant infectious agents that affects humankind. *S. mansoni* is a member of the Phylum Platyhelminthes and Family Schistosomatidae (a Family of digenetic trematodes) (Morgan *et al.*, 2001). Phylogenetic analyses suggest that *Schistosoma* parasites originated from Asia and travelled to Africa and later the Neotropics. In Africa, the progenitor gave rise to two species groups; the terminally spined egg species group and the laterally spined egg species group, with the latter containing *S. mansoni* (Morgan *et al.*, 2001).

It is estimated that about 85 million people are infected with *S. mansoni* in 54 countries. In addition to infecting humans, *S. mansoni* is also found in rodents and wild primates, particularly baboons. The abundance of *S. mansoni* is largely attributed to the widespread distribution of its snail intermediate hosts of the genus *Biomphalaria* that are widely distributed in Africa, the Neotropics and Asia (Colley *et al.*, 2014). Several studies have explored factors underlying the compatibility between *S. mansoni* with *Biomphalaria* snail hosts and concluded that both infectivity of the parasite and resistance to the parasite by *Biomphalaria* snails are genetically controlled (Adema *et al.*, 1997; Yoshino & Vasta, 1996; Richards *et al.*, 1992).

Prevention and control efforts in the fight against schistosomiasis normally involve preventive chemotherapy by praziquantel, an isoquinoline drug. However, it has been documented that multiple rounds of mass drug administration contribute to the reduction in efficacy of praziquantel (Crellen *et al.*, 2016). It has been established that *S. mansoni* can develop resistance and tolerance to praziquantel, raising concern about the future efficacy of the drug. *S. mansoni* populations with high genetic diversity have been shown to be resistant to praziquantel treatment (Faust *et al.*, 2019).

Migration facilitated by human hosts, as carriers of *Schistosoma* parasites could be a factor that contributes to the genetic diversity in *S. mansoni* populations. Mobility of human beings between different transmission foci has a role to play in the genetic structuring process of the schistosome populations; could increase geneflow among different parasite populations and reduce genetic differentiation among the populations (Dabo *et al.*, 1997). Long-lived adult worms in human beings also contribute to the genetic diversity of *S. mansoni* populations, by heightening the genetic diversity in populations where they are introduced.

Various studies have investigated the population genetic structure of *S. mansoni* in non-human hosts. Sire *et al.* (2001) analysed the genetic structure of *S. mansoni* recovered from *Rattus rattus* found in Guadeloupe with the revelation of high genetic diversity in the *S. mansoni* populations. A study by Minchella *et al.* (1995) on the population genetics of *S. mansoni* in *B. glabrata* snails revealed high levels of genetic variability of *S. mansoni* populations and a diverse array of parasite genotypes within sites; this was stated to play a role in increasing the infectivity of *S. mansoni*.

The primary objective of this study was to investigate the population genetics of *S. mansoni* in *Biomphalaria* snails found in East Africa's River systems. The study aims to contribute towards the body of knowledge on the population genetic structure of *S. mansoni*

populations, with the ultimate goal being to assist in coming up with appropriate intervention strategies, in the fight against schistosomiasis.

6.2 RESULTS

6.2.1 Phylogenetic tree

The phylogenetic tree was created in PhyML software version 2.4.4, using the GTR+G model, and with bootstrap values based on 1000 replicates. *S. japonicum* takes a basal position in the tree as shown in Figure 41. This suggests that *S. japonicum* is the oldest *Schistosoma* species. *S. mekongi* takes a closer position to *S. japonicum* suggesting that it is more closely related to *S. japonicum* when compared to the other *Schistosoma* species. *S. indicum* takes a closer position to *S. spindale* suggesting that the two species are closely related. *S. haematobium* and *S. curassoni* take a closer position to each other on the phylogenetic tree, suggesting that they are closely related. A significant amount of genetic variation can be deduced from the phylogenetic tree, from our *S. mansoni* from East Africa.

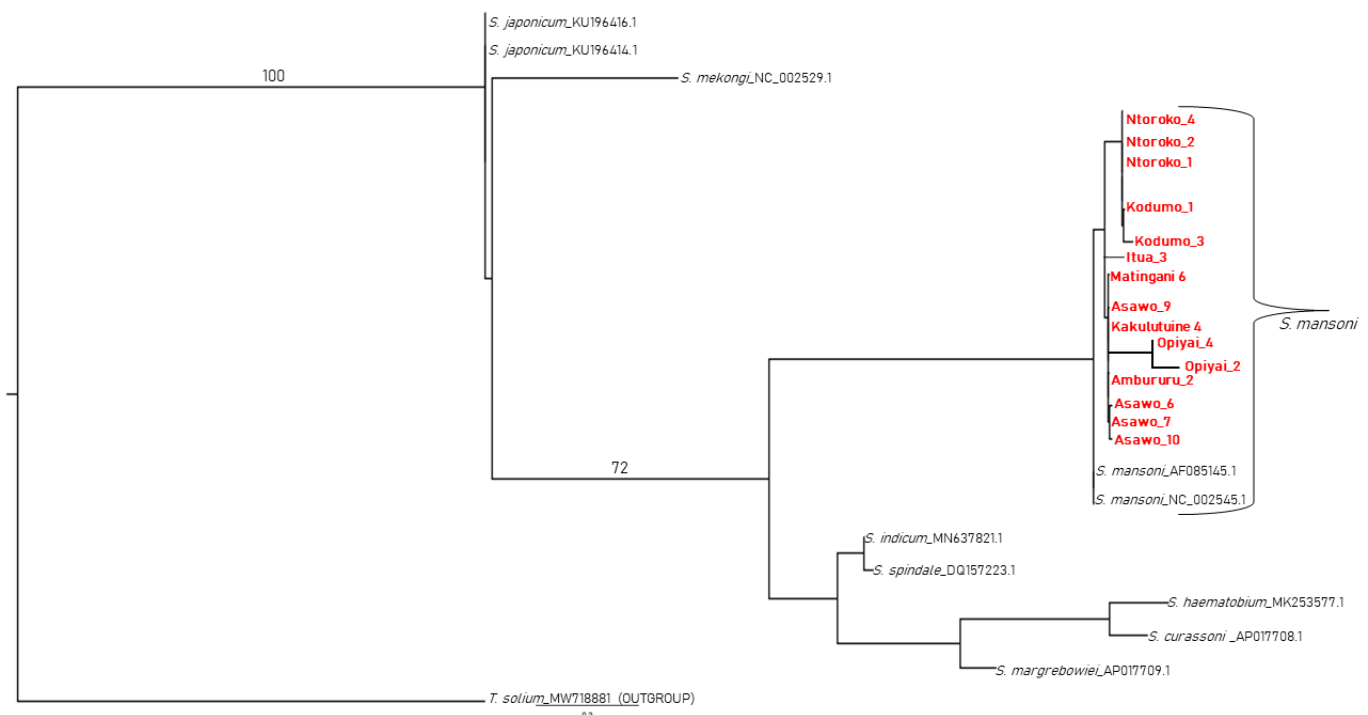


Figure 41: A Phylogenetic tree showing the NADH dehydrogenase subunit 5 gene relationship among several *Schistosoma* species. *S. mansoni* identified in our fieldwork study are highlighted in red.

6.2.2 Genetic analysis of *S. mansoni* ND5 sequence data

Genetic variation within populations and among populations was calculated by use of the AMOVA statistical method. DnaSP6 software was used to generate haplotype data information. A total of 16 ND5 haplotypes with a haplotype diversity of 1 and nucleotide diversity of 0.35891 were identified. Genetic diversity within populations was 53.34% and genetic differentiation among populations was 46.66% with an F_{ST} value of 0.46660 suggestive of moderate to high gene flow (Table 16).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	4	468.400	28.69632 V_a	46.66
Within populations	11	360.850	32.80455 V_b	53.34
Total	15	829.250	61.50087	

Table 16: Analysis of Molecular Variance of the schistosome ND5 mitochondrial gene data within and among pre-patent and sub-patent *S. mansoni* populations.

PopArt software version 1.7 was used to generate a minimum spanning haplotype network. The Minimum Spanning haplotype network generated in PopArt reveals the connection between the 16 ND5 haplotypes (Figure 42).

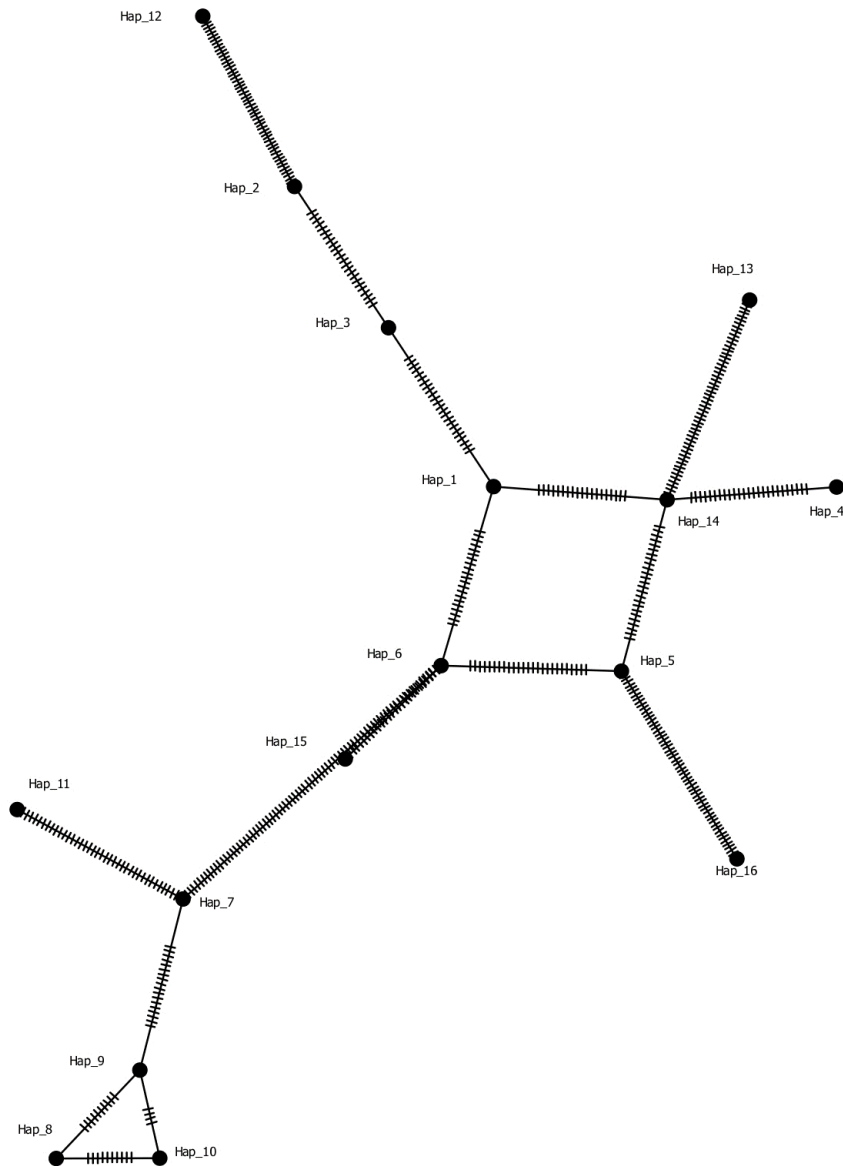


Figure 42: A minimum Spanning haplotype network generated using *S. mansoni*'s ND5 gene sequence data.

6.3 DISCUSSION

S. japonicum's basal position in the phylogenetic tree agrees with the theory that suggests that *Schistosoma* parasites originated from Asia and travelled to Africa from where *Schistosoma* parasites spread to the Neotropics (Lockyer *et al.*, 2003). *S. haematobium* and *S. curassoni* close position on the phylogenetic tree could be explained by the fact that both *S. haematobium* and *S. curassoni* are livestock schistosomes. The close relationship between *S. haematobium* and *S. curassoni* on the phylogenetic tree could explain cases of hybridization between the two species (Leger *et al.*, 2016). *S. spindale* and *S. indicum* close position to each other on the phylogenetic tree could be explained by the fact that they belong to the *Schistosoma indicum* group, they are widespread across South East Asia and they are both parasites of veterinary importance (Devkota *et al.*, 2015). A significant amount of genetic variation can be deduced from the phylogenetic tree, from our *S. mansoni* from East Africa. This agrees with the study by Morgan *et al.*, (2005) that discovered that *S. mansoni* populations from East Africa show a high level of mitochondrial differentiation when compared to that of *S. mansoni* populations from other regions of the world.

The population genetic structure of *Schistosoma* parasite populations has a role to play in their survival, infectivity, and resistance to selection pressures such as anti-schistosomal drugs (Rey *et al.*, 2021). There was revelation of moderate to high levels of genetic variation in *S. mansoni* populations (intrapopulation) as well as moderate to high levels of genetic differentiation among *S. mansoni* populations in *Biomphalaria* snails found in East Africa's River systems. A high level of heterogeneity was revealed by ND5 sequence data. This study corroborates with previous studies on the population genetic structure of *S. mansoni* in East Africa. A study by Agola *et al.* (2006) using microsatellite markers on Kenyan *S. mansoni* populations revealed that the parasites had high levels of genetic diversity within and between populations and concluded that limited gene flow is the probable cause of the strong population genetic structure. A study by Gower *et al.* (2013) documented high levels of genetic differentiation between Kenyan, Ugandan and Tanzanian *S. mansoni* populations characterised by moderate to high F_{ST} values.

There are several probable factors that might explain the high levels of genetic variation at intra and inter population levels including random mating, limited gene flow between populations and adaptability by *Schistosoma* parasites to new challenges and environments. Random mating (sexual reproduction) among individuals of genus Schistosomatidae is presumed to be advantageous, more so in cases where parents are genetically variable, meaning that offspring will exhibit all possible genotypic combinations of alleles of their parents. Among members of genus Schistosomatidae, sexual reproduction occurs in vertebrate hosts (Minchella *et al.*, 1995). *Schistosoma* parasites are regarded as atypical trematodes because when compared to other trematodes, they are dioecious (they have separate sexes) yet other trematodes are typically hermaphroditic (meaning that they mostly engage in non-random mating). Novel genotypic combinations that emerge courtesy of random mating are helpful in ensuring the offspring adapt to selection pressures, which could either be anthropogenic or natural, increasing their survival rates. Alternate sexual and asexual life cycle stages in mammalian and invertebrate hosts is also another strategy that is used by *Schistosoma* parasites to maintain high levels of genetic diversity within their populations. It has also been suggested

that human beings are 'genetic mixing bowls' for schistosomes. The 'genetic mixing bowls' concept means that human beings are often infected by different strains of *Schistosoma* parasites and inside human bodies, the different strains mix up leading to emergence of novel hybrid *Schistosoma* parasites (Van *et al.*, 2014).

There is evidence that suggests that *Schistosoma* species have the ability to quickly adapt to new challenges and new environments. The adaptability by *Schistosoma* parasites is meant to ensure their survival. The quick adaptability trait in *Schistosoma* parasites can only be achieved by the parasites heightening their genetic diversity. Therefore, it can be inferred that the high genetic diversity of *S. mansoni* populations in our study partly stems from *Schistosoma* parasite's innate ability to increase the genetic diversity of their populations to ensure their survival in an uncertain world.

Geneflow in *S. mansoni* populations could be instigated by migration of human beings harbouring the parasites, from one location to another. However, in Africa, geneflow by human mobility could be limited because school aged children, teenagers and women (who are most of the time carriers of schistosomes courtesy of their intense activities in snail infested water habitats) rarely migrate across divisions, districts, counties and provinces. Geographical distance between *S. mansoni* populations is a probable major cause of limited geneflow among the populations. It can be deduced from the results of this study that there is limited geneflow, subsequently leading to high levels of genetic structuring in *S. mansoni* populations harboured in the *Biomphalaria* snails. Furthermore, it has been established that high levels of population genetic structuring are likely to increase the likelihood in emergence of alleles responsible for conferring drug resistance.

In conclusion, results from this study affirm that there are moderate to high levels of genetic diversity in *S. mansoni* populations harboured in *Biomphalaria* snails found in East Africa's River systems. Furthermore, the study reveals moderate to high levels of genetic differentiation among *S. mansoni* populations. Results from this study could be interpreted as suggesting that the population genetic structure of *S. mansoni* parasites can potentially be a hindrance to the success of preventive chemotherapy efforts in East Africa because of the innate ability of the parasite to handle selection pressures courtesy of heightening the genetic diversity of its populations which makes it resistant to different selection pressures.

6.4 CONCLUSION

In conclusion, investigating the population genetics of *S. mansoni* populations is paramount because it can help us to understand the potential selection pressures that shape the population genetic structuring process of the said populations. It has been ascertained that the population genetic structure of *Schistosoma* parasites has a role to play in their survival, infectivity and resistance to antischistosomal drugs. In this study we investigated the population genetics of pre-patent and sub-patent *S. mansoni* populations using the ND5 mitochondrial gene. Genetic variation within our fieldwork *S. mansoni* populations was moderate, so was the genetic differentiation among the populations, suggestive of moderate gene flow. The moderate amount of genetic variation agrees with results of previous population genetics studies of *S. mansoni* in East Africa that allude to *S. mansoni* populations from East Africa showing a high level of mitochondrial DNA differentiation

when compared to *S. mansoni* populations from other regions of the world. Probable causes of the moderate levels of genetic variation are random mating, incidents of gene flow and the ability of *Schistosoma* parasites to quickly adapt to new challenges and environments. From a preventive chemotherapy perspective, it can be interpreted that the population genetic structure of *S. mansoni* parasites can potentially be a hindrance to the success of preventive chemotherapy efforts in East Africa because of the innate ability of the parasite to handle selection pressures courtesy of heightening the genetic diversity of its populations which makes it resistant to different selection pressures.

CHAPTER 7: CONCLUSION, REFLECTION AND FUTURE RESEARCH

7.1 CONCLUSION

In conclusion, the study aimed to elucidate the population genetic structure of *Biomphalaria* snails in East Africa's River systems, investigate the geographical distribution of the snails in the river systems, assess *S. mansoni* infection rates in the snails and explicate the population genetic structure of subpatent and prepatent *S. mansoni* populations. The methodological approach undertaken involved malacological surveys, DNA extraction, amplification of specific molecular markers (for population genetic studies and schistosome infection detection), visualisation of PCR products, DNA sequencing and genetic analysis. Genetic variation within *B. pfeifferi* populations was low whereas genetic variation within 'Nilotic Species Complex' populations was moderate. As for genetic differentiation among populations, there were high levels of genetic population differentiation among *B. pfeifferi* populations and moderate levels for 'Nilotic Species Complex' populations. The study revealed that *B. pfeifferi* is the most dominant *Biomphalaria* species in terms of geographical distribution in East Africa's River systems; 23/31 sites where *Biomphalaria* snails were found were inhabited by *B. pfeifferi* populations. There was low to moderate schistosome infection rates in the snails, ranging from 10% to 60% with all *Biomphalaria* populations that were found infected being *B. pfeifferi* snails; no *Biomphalaria* individual that belongs to the 'Nilotic Species Complex' was found infected. As for the population genetic structure of prepatent and subpatent *S. mansoni* populations, the populations exhibited moderate to high levels of intra and interpopulation genetic diversity.

7.2 REFLECTION

The results of this study suggest that locations in East Africa outside the Lake Victoria basin and the Albertine region should be prioritised in future studies of schistosomiasis in East Africa, as well as being included in the control intervention strategies. The low genetic variation within *B. pfeifferi* populations is suggestive of inbreeding whereas the moderate genetic variation within 'Nilotic Species Complex' populations is suggestive of incidences of cross breeding and gene flow. It was also established that morphological characters are not adequate to differentiate *Biomphalaria* snails accurately to the species level. Courtesy of molecular tools *B. pfeifferi* clade was established to be strictly monophyletic whereas members of the 'Nilotic Species Complex' intermingled on the phylogenetic trees. From the study, it can be inferred that *B. pfeifferi* is the most dominant *Biomphalaria* snail in terms of geographical distribution in East Africa's River systems. As far as *Biomphalaria* snails' habitats are concerned, streams appear to be more favourable to the habitation of the snails than rivers, irrigation schemes, dams or springs. In terms of schistosome infection detection, the results herein can be interpreted to mean that *S. mansoni* is more compatible with *B. pfeifferi* than other *Biomphalaria* species found in East Africa's River systems. Given that population genetic analysis revealed that most of the *B. pfeifferi* were generally homozygous, and they are the ones that were found to be infected with *S. mansoni* whereas members of the 'Nilotic Species Complex' were generally heterozygous and were not infected; this supports the hypothesis that lack of allelic richness in *Biomphalaria* populations increases success rates of parasitic infections. The moderate

levels of genetic variation within *S. mansoni* populations mean that there are incidences of migration, cross-fertilization, and gene flow among the populations. The moderate amount of genetic variation agrees with results of previous population genetics studies of *S. mansoni* in East Africa that allude to *S. mansoni* populations from East Africa showing a high level of mitochondrial DNA differentiation when compared to *S. mansoni* populations from other regions of the world.

The World Health Organization's strategy towards elimination of schistosomiasis as a public health problem by 2030 includes preventive chemotherapy, improved access to drinking water, improved sanitation, hygienic education, environmental management, and snail control. Kenya has a 2023 – 2037 masterplan for the elimination of neglected tropical diseases. The strategy involves vector control, public health education, provision of safe water and improved sanitation. Uganda has a 2023 – 2027 masterplan to eliminate Neglected Tropical Diseases. It is highlighted in the Uganda plan that schistosomiasis is endemic to 96 districts out of the total 135 districts. Tanzania has a 2021– 2026 strategic plan to eliminate schistosomiasis as a public health problem. The strategic plan involves the continued mapping of schistosomiasis endemic areas and the populations at risk as well as conducting mass drug administration programs in the endemic areas.

This thesis provides useful information to understand the taxonomy, infection biology, and options for snail control in East Africa. It was ascertained that the use of morphological characters such as shell morphology is not reliable in the classification of *Biomphalaria* snails to the species level. We established that ecological factors play a major role in the development of shell size, aperture size and whorls of the shells, resulting to shell plasticity thus making morphology an unreliable parameter in the classification of the snails. Use of molecular tools was found to be more reliable in the classification of *Biomphalaria* snails in East Africa to the species level. *B. pfeifferi* was clearly classified by molecular means; however, *B. choanomphala*, *B. sudanica*, *B. stanleyi*, *B. smithi* and other *Biomphalaria* species in East Africa River systems could not be clearly identified and were all treated as members of the 'Nilotic Species Complex.' This thesis also contributes to the body of knowledge on the topic of infection biology, regarding *Schistosoma* parasites and snail intermediate hosts. The thesis provides information that supports the hypothesis that the genetic structure of freshwater snails has a role to play in the success of parasitic infections in the snails. All the *Biomphalaria* populations that were found infected with *S. mansoni* in this study were those that were generally homozygous (exhibited low levels of intrapopulation genetic diversity). The use of molluscicides, especially plant molluscicides because of their low toxicity towards non – target hosts is the approach that we do propose regarding the control of snail intermediate hosts, as an intervention approach towards controlling the transmission of human schistosomiasis in line with the 2030 WHO control targets. WHO recognises interruption of transmission as a vital approach towards schistosomiasis control and in this case, by eliminating the snail intermediate host, we will stop the development of asexual stages of *Schistosoma* parasites and there will be no release of cercaria which infect humans. The use of reliable molecular markers in the detection of schistosome infections in animal reservoirs, in snail hosts and in humans is one of the strategies recommended towards attainment of the 2030 WHO control targets. In this study we used an ND5 gene marker and a 28S rDNA region marker to detect *S.*

mansoni infections. The primers we used are reliable, sufficient and were proven to be *S. mansoni* specific by acquisition and verification of DNA sequences. We were able to differentiate *S. mansoni* from *S. rodhaini* infections courtesy of the verification of the DNA sequences.

7.3 FUTURE RESEARCH

Future work can include conducting cercarial shedding studies on snails from sites where the snails were found. Recommendation 6 of the newly revised WHO guidelines for control and elimination of schistosomiasis states that in communities approaching the interruption of schistosomiasis transmission, a verification framework should be established that tests *Schistosoma* infection in humans, snails and non-human mammalian hosts with a two-step diagnostic process that exudes high sensitivity and specificity (WHO, 2022). Cercarial shedding can be included as one of the diagnostic tests that exudes high sensitivity and specificity and tests for schistosome infection in snails.

Future work can also include conducting diagnostic tests in human subjects inhabiting communities next to where the snails were found. Given that Recommendations 1, 2 and 3 of the newly revised WHO guidelines for the control and elimination of schistosomiasis recommend annual preventive chemotherapy in endemic communities with an infection rate of at least 10% after the prevalence threshold has been ascertained by Kato-Katz smears (WHO, 2022); it will be prudent if future work as an extension of this study conducts Kato-Katz tests among human communities in close proximity to the habitats where *Biomphalaria* populations infected with *S. mansoni* were found.

Future work can also include use of molluscicides as part of an integrated approach for schistosomiasis control in locations where *Biomphalaria* snails were found. Recommendation 5 of WHO for control and elimination of schistosomiasis (WHO, 2022) recommends WASH (Water, Sanitation and Hygiene) interventions, environmental interventions (water engineering and focal snail control with molluscicides) and behavioural change interventions as essential measures to help reduce transmission of schistosomes in endemic areas. Future work can also include visiting regions where sampling was not properly done to ascertain whether more *Biomphalaria* populations can be found.

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APPENDIX

Supplementary Table 1: GBIF Occurrence Records table

GBIF OCCURRENCE RECORDS IN A TABLE

COUNTRY	GBIF Catalogue Number/Occurrence ID	GEOGRAPHIC COORDINATES
Angola	NHMUK:ecatalogue:8168749	(-9.300000, 16.100000)
Benin	ET_MBI_1559	(10.200000, 2.100000)
Benin	ET_MBI_1563	(6.400000, 2.200000)
Benin	2019-10	(9.300000, 2.600000)
Bostwana	19000	(-18.300000, 21.800000)
Bostwana	10283	(-18.400000, 21.900000)
Bostwana	9035	(-18.400000, 22.000000)
Bostwana	52010	(-18.900000, 22.400000)
Bostwana	56691	(-19.000000, 22.400000)
Bostwana	59700	(-19.100000, 23.400000)
Bostwana	28139	(-19.200000, 23.200000)
Bostwana	64191	(-19.500000, 24.100000)
Botswana	52010	(-18.900000, 22.400000)
Botswana	21221	(-19.500000, 23.100000)
Burkina Faso	MSB:Host:20665	(11.800000, -0.500000)
Burundi	NHMUK:ecatalogue:8168777	(-3.000000, 29.400000)
Burundi	NHMUK:ecatalogue:8168772	(-4.200000, 29.600000)
Burundi	NHMUK:ecatalogue:8168778	(-3.000000, 29.400000)
Cameroon	BE_RMCA_MOL.Gas.804806	(4.500000, 9.300000)
Cameroon	NHMUK:ecatalogue:8173798	(3.900000, 11.500000)
Chad	NHMUK:ecatalogue:9178255	(19.100000, 20.500000)
Chad	NHMUK:ecatalogue:8168973	(12.100000, 15.100000)

Congo	6610884	(-1.500000, 29.300000)
Congo	132860	(-7.000000, 23.900000)
Congo	BE_RMCA_MOL.Gas.804817	(0.500000, 25.200000)
Congo	186459	(-7.000000, 23.900000)
Cote d'Ivoire	CNFD0B020849	(5.200000, -2.900000)
Egypt	MSB:Host:20664	(29.700000, 31.200000)
Egypt	558617-Mollusca	(25.700000, 32.600000)
Kenya	MSB:Host:25220	(0.100000, 34.700000)
Kenya	MSB:Host:20705	(-0.100000, 34.700000)
Kenya	MSB:Host:22342	(0.200000, 34.400000)
Kenya	MSB:Host:22300	(-0.300000, 35.000000)
Kenya	MNMK1064BP9	(-0.400000, 34.200000)
Kenya	MSB:Host:24891	(-0.300000, 35.200000)
Kenya	MNMK1064BP8	(0.600000, 34.500000)
Kenya	MNMK1064BP10	(0.700000, 34.500000)
Kenya	MNMK1064BP21	(-1.200000, 36.900000)
Kenya	MNMK1064BP11	(-1.300000, 37.300000)
Kenya	MNMK1064BP22	(-1.400000, 36.700000)
Kenya	MNMK1064BP23	(-2.700000, 37.300000)
Kenya	MNMK1064BP6	(3.300000, 37.100000)
Kenya	MNMK1075BS3	(-0.100000, 36.400000)
Kenya	MSB:Host:25074	(0.200000, 34.300000)
Kenya	MSB:Host:24905	(-0.300000, 35.000000)
Malawi	RMNH.MOL.511026	(-13.500000, 34.000000)
Malawi	DMNS:Inv:38264	(-15.800000, 35.000000)
Niger	6619408	(19.000000, 10.500000)
Nigeria	MSB:Host:20673	(9.300000, 9.600000)

Senegal	GBML27974-19	(14.200000, -14.600000)
Senegal	172740	(14.700000, -17.500000)
Senegal	NHMUK:ecatalogue:8535873	(16.100000, -16.400000)
Senegal	NHMUK:ecatalogue:8171940	(16.500000, -15.600000)
Senegal	GBML27974-19	(14.200000, -14.600000)
Senegal	BE_RMCA_MOL.Gas.804931	(16.400000, -15.900000)
Senegal	BE_RMCA_MOL.Gas.804949	(16.500000, -15.900000)
Senegal	NHMUK:ecatalogue:8534742	(15.900000, -15.900000)
Senegal	NHMUK:ecatalogue:8535404	(16.100000, -16.400000)
Senegal	NHMUK:ecatalogue:8173479	(16.300000, -15.800000)
Senegal	NHMUK:ecatalogue:8173565	(16.400000, -15.700000)
South Africa	LIM 893Z	(-23.800000, 31.600000)
South Africa	GEN 155K	(-25.100000, 30.800000)
South Africa	ELMD06212	(-32.200000, 29.000000)
Tanzania	MNMK1075BS15	(-2.500000, 32.900000)
Tanzania	84352	(-3.100000, 37.400000)
Tanzania	GBML27890-19	(-6.400000, 34.800000)
Tanzania	NHMUK:ecatalogue:8536591	(-2.700000, 32.800000)
Tanzania	NHMUK:ecatalogue:8536623	(-2.300000, 32.200000)
Tanzania	NHMUK:ecatalogue:8172704	(-2.800000, 32.900000)
Tanzania	MNMK0408BC11	(-2.100000, 32.900000)
Tanzania	159078	(-2.500000, 32.900000)
Tanzania	NHMUK:ecatalogue:8536489	(-2.500000, 32.800000)
Tanzania	NHMUK:ecatalogue:8536295	(-2.500000, 32.800000)
Tanzania	NHMUK:ecatalogue:8536370	(-2.700000, 32.800000)
Tanzania	NHMUK:ecatalogue:8172605	(-2.400000, 32.100000)
Uganda	RS_NaFIRRI_Macro Inverts_12346	(0.100000, 32.600000)

Uganda	BE_RMCA_MOL.Gas.804913	(-0.800000, 30.900000)
Uganda	GBML27983-19	(1.300000, 32.300000)
Uganda	BE_RMCA_MOL.Gas.804543	(-0.300000, 30.100000)
Uganda	118593	(-0.300000, 32.600000)
Uganda	159084	(0.800000, 30.100000)
Uganda	GBML27983-19	(1.300000, 32.300000)
Uganda	172097	(1.800000, 31.700000)
Uganda	124545	(1.800000, 31.300000)
Uganda	171168	(0.000000, 30.100000)
Uganda	171716	(-0.400000, 29.600000)
Uganda	BE_RMCA_MOL.Gas.804500	(0.400000, 30.200000)
Uganda	BE_RMCA_MOL.Gas.804552	(0.400000, 30.300000)
Uganda	RS_NaFIRRI_Macro Inverts_443	(0.400000, 33.200000)
Uganda	BE_RMCA_MOL.Gas.804567	(0.400000, 33.300000)
Uganda	137126	(1.800000, 31.300000)
Uganda	BE_RMCA_MOL.Gas.804871	(-0.500000, 31.200000)
Uganda	BE_RMCA_MOL.Gas.804479	(0.700000, 30.300000)
Uganda	BE_RMCA_MOL.Gas.804870	(-0.800000, 30.900000)
Uganda	RS_NaFIRRI_Macro Inverts_15370	(1.800000, 31.300000)
Uganda	171384	(-1.200000, 29.700000)
Uganda	171383	(0.300000, 32.600000)
Uganda	41128	(0.400000, 29.900000)
Uganda	GBMLG9911-13	(1.300000, 32.300000)
Zimbabwe	BE_RMCA_MOL.Gas.804643	(-17.200000, 31.000000)
Zimbabwe	BE_RMCA_MOL.Gas.804634	(-17.300000, 31.000000)

Supplementary Figure 1: GBIF Occurrence Records on a map



Supplementary Table 2: Name of Sampled sites with associated parameters

Name of site	Coordinates	Altitude	Type of habitat	<i>Biomphalaria</i> Snails found (Yes/No)	Water Temp (°C)	pH	Soil Type	Veg (Y/N)
Mbondoni dam	(-0.97325, 38.005)	1121m	Dam	No	31.2	7.3	Sand soil	N
Migwani dam	(-1.09305556, 38.02111)	1116m	Dam	No	29.1	6.2	Sand soil	N
Matingani seepage	(-1.164, 38.005)	1184m	Stream	Yes	23.2	5.2	Clay soil	Y
Kangonde dam	(-1.07941667, 37.69166667)	1183m	Dam	No	31.2	8.3	Sand soil	N
Mutendea stream	(-1.33180556, 37.98166667)	1098m	Stream	No	29.1	7.3	Sand soil	Y
Kiangangi irrigation canal	(-0.593833, 37.341972)	1365m	Irrigation scheme	Yes	24.5	8.2	Silt soi	Y
Kitui stream	(-1.35616667, 38.00611111)	1166m	Stream	Yes	20.6	6.4	Silt soil	Y
Kalundu stream A	(-1.35616667, 38.00611111)	1216m	Stream	Yes	20.4	7.3	Sand soil	Y
Kalundu stream B	(-1.36555556, 38.00277778)	1214m	Stream	Yes	20.1	8.2	Sand soil	Y
Ikindu stream	(-1.36733333, 38.03916667)	1215m	Stream	No	31.2	9.2	Sand soil	Y
Nzeu stream	(-1.37061111, 38.02416667)	1138m	Stream	No	29.1	7.2	Sand soil	N
Kwase stream	(-1.29883333, 37.35722222)	1568m	Stream	Yes	19.3	6.8	Silt soil	Y
Mutanga Ivutu stream	(-1.35758333, 37.35472222)	1484m	Stream	Yes	25.7	8.2	Silt soil	Y
Chumbe stream A	(-2.26511111, 37.81027778)	950m	Stream	No	32.1	7.9	Sand soil	N
Ikoyo Dam	(-2.26613889, 37.81)	994m	Dam	No	31.2	8.4	Sand soil	N

Thange River	(-2.50158333, 38.02277778)	777m	River	No	15.6	7.3	Sand soil	Y
Thange River 2	(-2.47536111, 38.09527778)	775m	River	No	15.2	8.3	Sand soil	Y
Kambu River 1	(-2.536591, 38.118119)	781m	River	No	31.5	7.4	Sand soil	Y
Kambu River 2	(-2.498523, 38.053190)	782m	River	No	30.9	9.3	Sand soil	Y
Loilopon	(-2.930847, 37.476999)	1910m	Stream	No	13.2	8.2	Silt soil	N
Kambu river 3	(-2.56630556, 38.11944444)	779m	River	No	20.9	9.3	Sand soil	Y
Itua River	(-0.62888889, 37.54)	653m	River	Yes	25.4	8.3	Silt soil	Y
Thiba river	(-0.77269444, 37.63888889)	1009m	River	No	15.3	7.2	Sand soil	Y
Tulimumbu stream	(-0.91133333, 37.65833333)	1007m	Stream	Yes	21.3	6.8	Sand soil	Y
Kitunene Dam	(-0.92533333, 37.67583333)	1221m	Dam	No	15.2	9.2	Sand soil	N
Musilili stream	(-1.45023611, 37.2575)	1330m	Stream	Yes	24.6	8.3	Silt soil	Y
Kakulutuine stream	(-1.20472222, 37.33055556)	1188m	Stream	Yes	25.3	7.6	Silt soil	Y
Mukou stream	(-1.68488889, 37.34472222)	1319m	Stream	Yes	25.9	9.2	Silt soil	Y
Mtito River	(-2.68713889, 38.16)	464m	River	No	21.2	8.1	Sand soil	Y
Kahoma stream	(-3.42088889, 37.68305556)	845m	Stream	No	15.4	8.2	Silt soil	Y
Voi River	(-3.39063889, 38.57861111)	546m	River	No	13.2	6.4	Sand soil	N
Jipe A	(-3.59861111, 37.77444444)	705m	Stream	No	31.5	7.3	Sand soil	Y
Jipe B	(-3.61477778, 37.77611111)	706m	Stream	No	32.1	8.2	Sand soil	Y
Jombo River	(-3.50175, 38.37777778)	903m	River	No	14.2	7.1	Sand soil	N

Kasabong stream	(-0.15608333, 34.40583333)	1182m	Stream	No	29.3	8.3	Silt soil	N
Abitha stream	(-0.16761111, 34.40583333)	1184m	Stream	No	29.7	9.1	Silt soil	Y
Gera river 1	(-0.46413889, 34.22916667)	1152m	River	No	31.2	8.2	Silt soil	Y
Nyabanda dam	(-0.08961111, 34.28111111)	1281m	Dam	No	15.3	9.1	Sand soil	Y
Manyonge stream	(-0.06366667, 34.29166667)	1289m	Stream	No	14.2	7.8	Silt soil	Y
Nyawita stream	(-0.43036111, 34.15472222)	1149m	Stream	No	13.5	9.2	Silt soil	Y
Kosena Dam	(0.09338889, 34.28)	1281m	Dam	No	15.3	8.1	Sand soil	N
Ambururu stream	(0.15163889, 34.27916667)	1236m	Stream	Yes	23.5	7.5	Silt soil	Y
Mbita	(-0.42180556, 34.20611111)	1168m	River	No	29.3	6.3	Sand soil	Y
Thogoye Usenge	(-0.06091667, 34.04305556)	1134m	River	No	31.2	7.2	Sand soil	Y
Gera River	(-0.44213889, 34.22666667)	1198m	River	No	30.4	8.3	Sand soil	Y
Luanda Gembe stream	(-0.46413889, 34.22916667)	1172m	Stream	No	29.2	7.5	Silt soil	Y
Kulo Kudongo stream	(0.27072222, 34.19083333)	1221m	Stream	No	31.3	6.9	Silt soil	Y
Wathi River	(0.30513889, 34.23472222)	1292m	River	No	30.5	7.2	Sand soil	Y
Rakite stream	(0.32575, 34.19472222)	1285m	Stream	Yes	21.2	8.2	Silt soil	Y
Matodo stream	(0.30847222, 34.22972222)	1261m	Stream	No	31.3	7.3	Silt soil	Y
Nyatho stream	(-0.179, 35.07306)	1293m	Stream	No	29.1	7.3	Silt soil	Y
Asawo River	(-0.31817, 35.007)	1232m	River	Yes	24.5	6.8	Silt soil	Y

River Awach	(-0.23405, 34.95717)	1278m	River	No	29.2	7.2	Sand soil	N
Awach river B	(-0.27203, 35.00374)	1273m	River	No	29.7	6.4	Sand soil	N
Asao river A	(-0.29416, 34.94199)	1255m	River	No	30.1	7.2	Sand soil	N
Asao river B	(-0.23403, 34.95717)	1327m	River	No	31.2	7.5	Sand soil	N
Agoro stream	(-0.27203, 35.00374)	1225m	Stream	No	30.6	7.5	Silt soil	N
River Nyando	(-0.17244, 34.92066)	1155m	River	No	31.2	7.3	Sand soil	N
Ahero rice field	(-0.17656, 34.92103)	1153m	Irrigation scheme	No	30.6	7.3	Silt soil	Y
Miriu river	(-0.39753, 35.01759)	1145m	River	No	31.3	7.6	Sand soil	N
Omondo stream	(-0.30537, 34.93082)	1142m	Stream	No	30.6	7.3	Silt soil	Y
Ochuoga stream	(-0.30075, 34.9295)	1141m	Stream	No	31.3	7.6	Silt soil	Y
Kosele stream	(-0.44471, 34.68103)	1330m	Stream	No	29.8	7.4	Silt soil	Y
Pundo stream	(-0.50641, 34.73087)	1410m	Stream	No	15.3	6.3	Silt soil	Y
Kodumo stream	(-0.41068, 34.99455)	1532m	Stream	Yes	21.2	7.3	Silt soil	Y
Kodongo stream	(-0.444710, 34.681030)	1548	Stream	Yes	16.2	6.9	Silt soil	Y
Katengu stream	(-0.39756, 35.00266)	1547m	Stream	No	15.2	7.3	Silt soil	Y
K'otieno stream	(-0.51078, 34.71803)	1544m	Stream	No	13.2	7.2	Silt soil	Y
Onsando Dam	(-0.71103, 35.0479)	1926m	Dam	Yes	25.4	6.7	Silt soil	Y
Siwot Dam	(-0.87596, 35.3683)	2166m	Dam	No	15.2	7.2	Sand soil	N

Cheptuyet stream	(-0.91062, 35.34843)	1914m	Stream	Yes	25.3	8.3	Silt soil	Y
Kabere stream	(0.41284, 33.49619)	1156m	Stream	Yes	19.1	9.2	Silt soil	Y
Kibimba irrigation scheme	(0.52956, 33.85815)	1080m	Irrigation scheme	Yes	24.7	7.3	Silt soil	Y
Macheche dam	(0.39348, 33.48281)	1134m	Dam	No	31.2	8.1	Sand soil	N
Kaitambiri stream	(1.12788, 33.70906)	1083m	Stream	No	15.2	8.4	Silt soil	Y
Nakitende shore	(1.13265, 33.67626)	1043m	Stream	No	14.2	7.6	Sand soil	Y
Walukuba stream	(0.44258, 33.22391)	1139m	Stream	Yes	25.6	7.3	Silt soil	Y
Ariet dam	(1.16151, 33.72042)	1073m	Dam	No	15.2	7.3	Sand soil	Y
Bisina	(1.59835, 33.95884)	1043m	River	No	14.2	7.4	Sand soil	Y
Opiyai A Angorom	(1.70238, 33.62261)	1119m	Spring	Yes	25.2	6.2	Silt soil	Y
Amidakan dam	(1.93723, 33.34933)	1043m	Dam	No	16.2	6.5	Sand soil	N
Kachung shore	(1.89908, 32.97164)	1045m	River	No	15.2	7.4	Sand soil	N
Masindi port	(1.69541, 32.09238)	1034m	River	Yes	19.2	6.7	Silt soil	Y
River Kole	(2.302313, 32.68811)	1056m	River	Yes	21.3	6.3	Silt soil	Y
Masaka stream	(-0.33681, 31.71796)	1249m	Stream	No	15.3	6.2	Silt soil	Y
Kagadi swamp	(0.94023, 30.81403)	1146m	Swamp	No	13.2	8.2	Silt soil	Y
Panyango shore	(2.52544, 31.46533)	615m	River shore	No	31.2	7.3	Silt soil	Y

Muzizi river	(0.87095, 30.72997)	1178m	River	Yes	21.1	9.2	Silt soil	Y
Ntoroko	(1.05375, 30.53696)	631m	River	Yes	20.8	8.4	Silt soil	Y
Lubiri	(2.45857, 31.49975)	632m	River	Yes	22.1	7.3	Silt soil	Y
Rwizi river	(-0.61686, 30.66828)	1480m	River	No	16.2	7.2	Sand soil	Y
Mpanga river	(0.658889, 30.273528)	1521m	River	No	15.2	7.3	Silt soil	Y
Kasese river	(0.17418, 30.08111)	925m	River	No	17.2	7.1	Silt soil	Y
Wia Amwon	(2.2595, 32.87927)	1100m	Stream	No	15.2	7.2	Silt soil	Y
Mapea irrigation scheme	(-3.99006, 35.73814)	962m	Irrigation scheme	No	13.1	6.8	Silt soil	Y
Njoro swamp	(-3.32104, 37.27832)	710m	Swamp	No	16.3	7.3	Silt soil	Y
Njoro forest stream	(-3.32598, 37.27805)	713m	Stream	No	14.3	6.1	Silt soil	Y
Rao river	(-3.3381, 37.35776)	715	River	No	15.2	8.5	Silt soil	Y
Kikavu irrigation scheme	(-3.43637, 37.29482)	702m	Irrigation scheme	No	16.2	8.2	Sand soil	Y
Mabogini irrigation canal	(-3.40061, 37.36256)	684m	Irrigation scheme	Yes	24.3	8.8	Silt soil	Y
Chiwe river	(-6.21983, 36.74357)	1249m	River	No	15.2	7.3	Sand soil	Y
Ihanda stream	(-6.24869, 36.71776)	1329m	Stream	No	15.2	7.2	Silt soil	Y
Mhango stream	(-6.24053, 37.5274)	1015m	Stream	No	14.2	8.5	Silt soil	Y
Mvomero river	(-6.30143, 37.44623)	1003m	River	No	16.2	8.2	Sand soil	Y

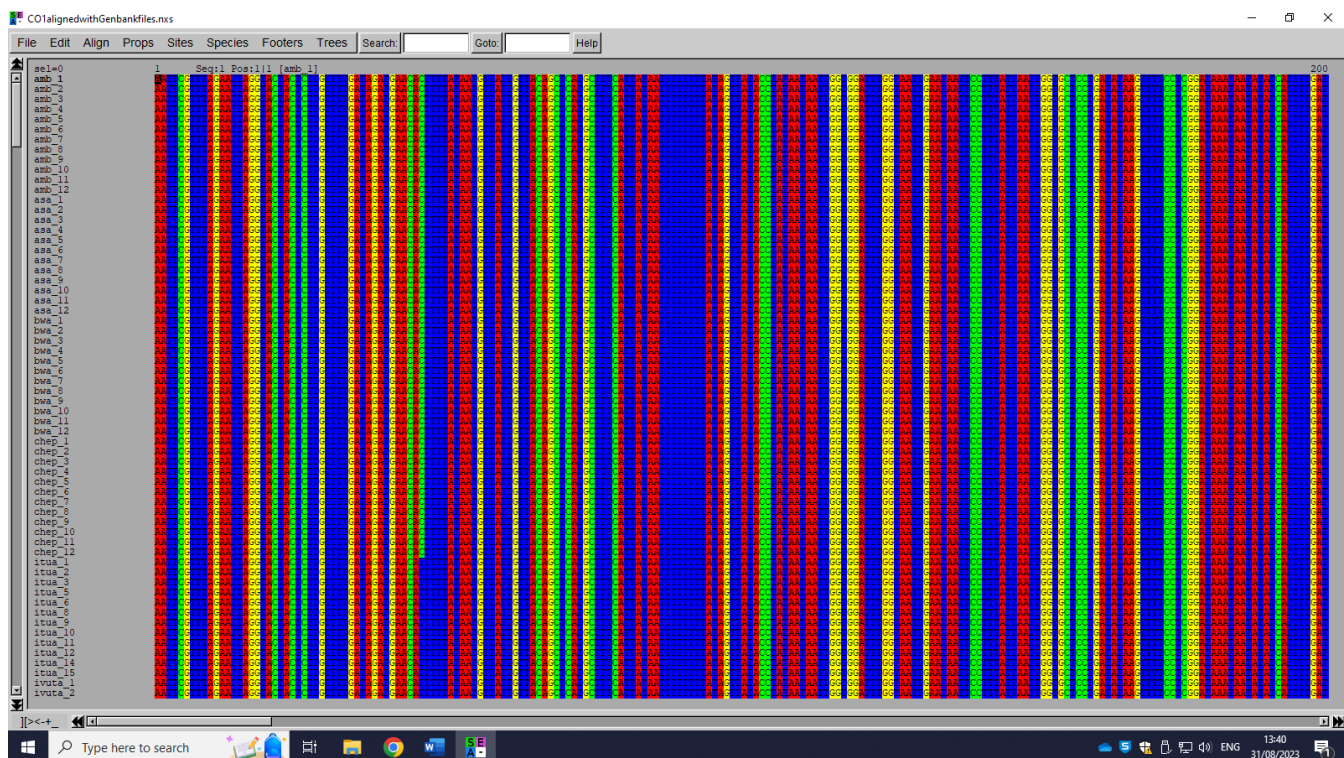
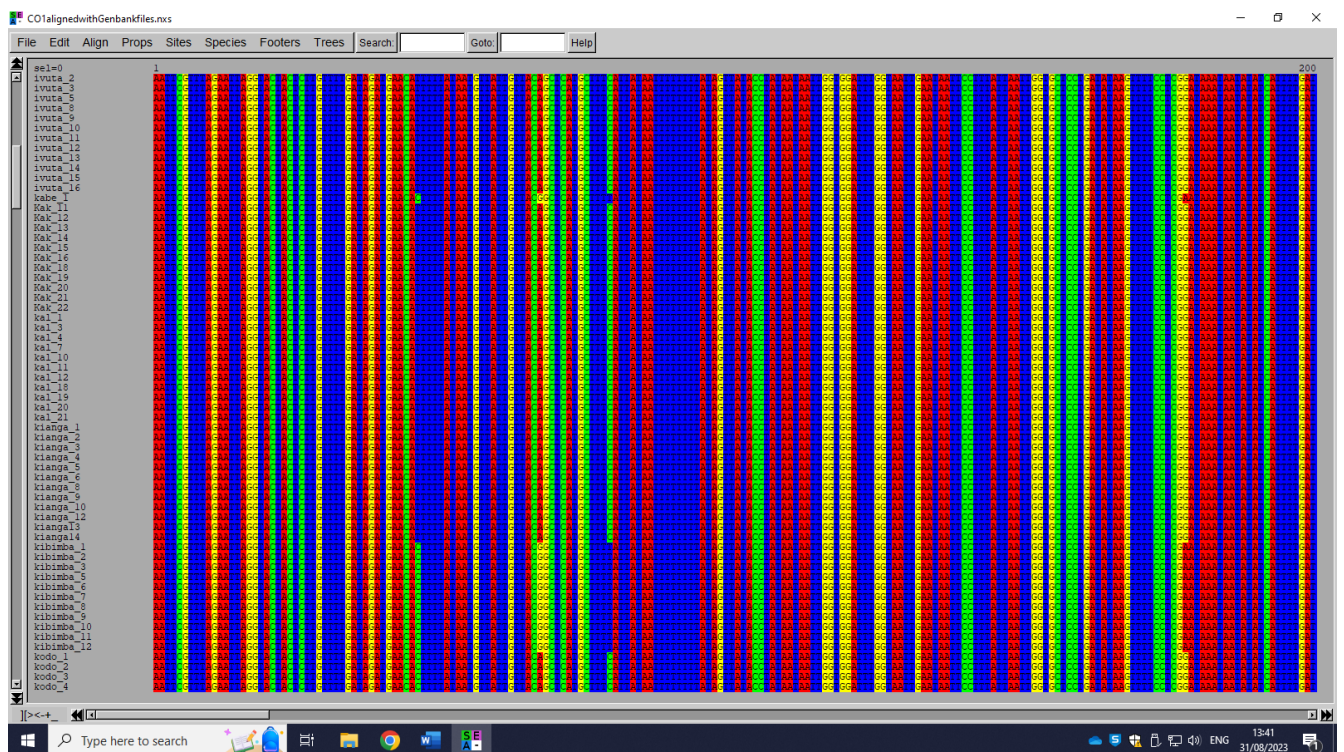
Mkindu river	(-6.24803, 37.54699)	1114m	River	No	14.2	7.5	Sand soil	N
Mkindo irrigation scheme	(-6.26084, 37.55375)	977m	Irrigation scheme	No	15.2	7.2	Silt soil	Y
Mlegeni stream	(-6.89333, 37.07764)	476m	Stream	No	16.3	7.6	Silt soil	Y
Dago stream	(-6.8934, 37.07775)	481m	Stream	No	15.3	8.1	Silt soil	Y
Zombo irrigation scheme	(-6.96513, 36.91566)	533m	Irrigation scheme	No	16.2	8.5	Silt soil	Y
Tindiga irrigation	(-6.89507, 37.0883)	459m	Irrigation scheme	No	16.2	8.2	Silt soil	Y
Chabi irrigation scheme	(-7.31147, 36.52885)	576m	Irrigation scheme	No	16.3	8.7	Silt soil	Y
Mwega irrigation scheme	(-7.31152, 36.52878)	660m	Irrigation scheme	No	13.2	8.1	Silt soil	Y
Bwawani, Iringa	(-7.73513, 35.71792)	1530m	Dam	Yes	24.1	8.5	Silt soil	Y
Ilongo irrigation scheme	(-8.76628, 33.74477)	1519m	Irrigation scheme	No	17.2	7.1	Silt soil	Y
Nsalagah stream	(-8.88944, 33.57027)	1813m	Stream	No	16.3	7.6	Silt soil	Y
Migombani stream	(-9.31781, 32.76955)	1654m	Stream	No	32.2	7.3	Silt soil	Y
Kasekera stream	(-4.689278, 29.622083)	1128m	Stream	No	31.2	7.2	Silt soil	Y
Singida	(-4.788596, 34.745608)	1388m	River	No	31.2	7.5	Sand soil	N
Kibilizi, Kigoma	(-4.860920, 29.624456)	767m	Stream	Yes	26.1	7.3	Silt soil	N
Musoma	(-1.494590, 33.810970)	1134m	River	No	29.2	6.5	Sand soil	Y
Bweri stream	(-1.537530, 33.854760)	1250m	Stream	No	14.7	6.9	Silt soil	Y

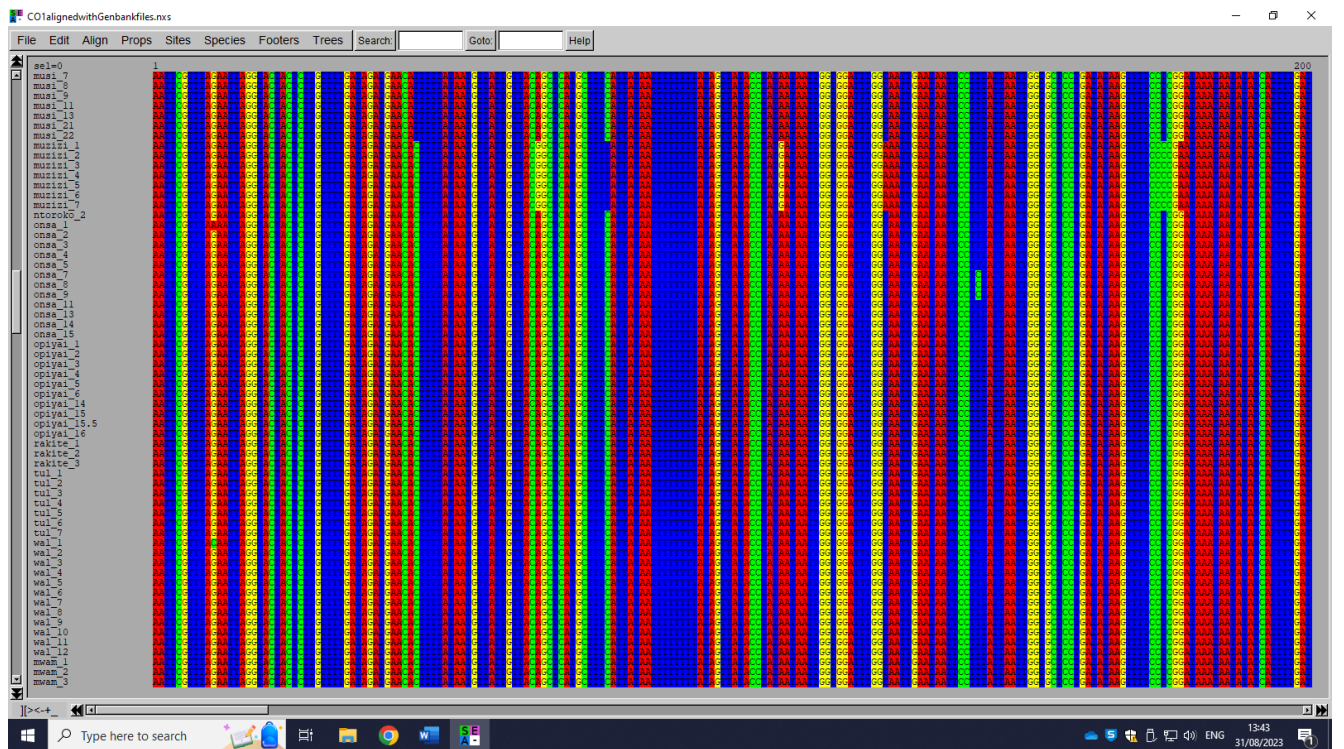
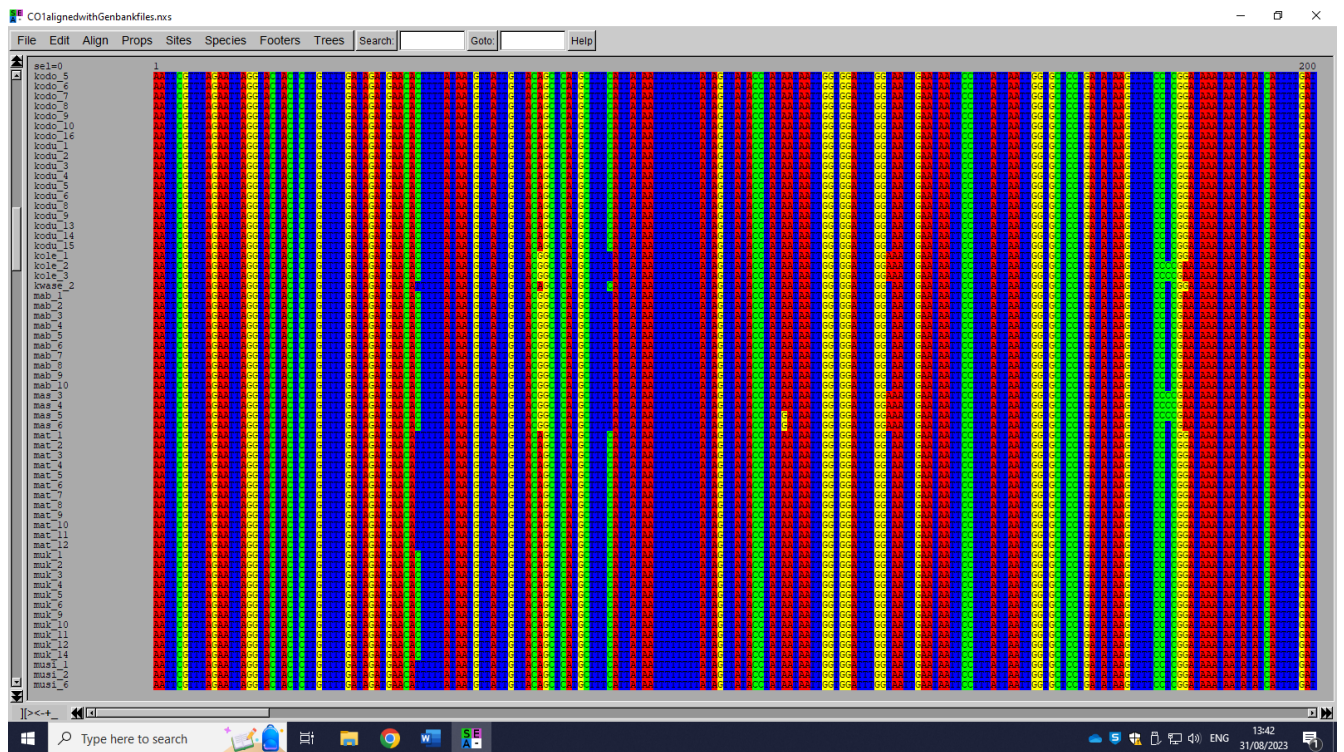
Ziro ziro stream	(-1.598370, 33.909480)	1214m	Stream	No	31.2	7.2	Silt soil	Y
Musoma road	(-1.414650, 34.213500)	1350m	Stream	No	29.2	7.5	Sand soil	Y
Kongoro, Tarime	(-1.339650, 34.385780)	1449m	Stream	No	15.8	6.9	Sand soil	Y
Suguti	(-1.680613, 33.703669)	1197m	River	No	16.5	7.1	Sand soil	Y
Nansimo	(-2.157424, 33.443241)	1141m	River	No	16.3	7.9	Sand soil	Y
Lamadi	(-2.246043, 33.836968)	1140m	River	No	15.3	7.2	Sand soil	Y
Mwamanyili	(-2.372647, 33.563318)	1155m	River	No	14.3	7.1	Silt soil	Y
Igogo	(-2.539848, 32.901068)	1134m	River	No	29.3	6.8	Silt soil	Y
Mnyuzi stream	(-5.305361, 38.628585)	230m	Stream	No	31.2	6.5	Silt soil	Y
Utwigu stream	(-4.446061, 33.050964)	1227m	Stream	Yes	20.2	6.3	Silt soil	Y
Chigunga shore	(-2.778439, 32.034190)	1139m	River	No	14.2	6.5	Sand soil	Y
Igombe dam	(-4.888247, 32.754505)	1149m	Dam	No	28.3	7.2	Sand soil	N
Mwaseni	(-7.792478, 38.094281)	64m	River	No	17.2	7.5	Sand soil	Y
Songea	(-10.662715, 35.56577)	932m	Dam	No	15.5	6.9	Sand soil	Y
Masonya	(-10.6992, 37.356541)	527m	River	No	15.4	8.1	Sand soil	Y
Shanwe stream	(-6.32568, 31.052803)	969m	Stream	No	15.2	7.3	Silt soil	Y
Sangari	(-5.17815, 31.15624)	1058m	River	No	16.3	6.5	Silt soil	N
Ntalikwa stream	(-5.07492, 32.71561)	1147m	Stream	No	17.3	7.4	Sand soil	Y
Ntalikwa rice field	(-5.0742, 32.71541)	1140m	Irrigation scheme	No	16.7	7.1	Silt soil	Y
Sumbawanga stream	(-7.99843, 31.61841)	846m	Stream	No	15.6	6.7	Silt soil	Y

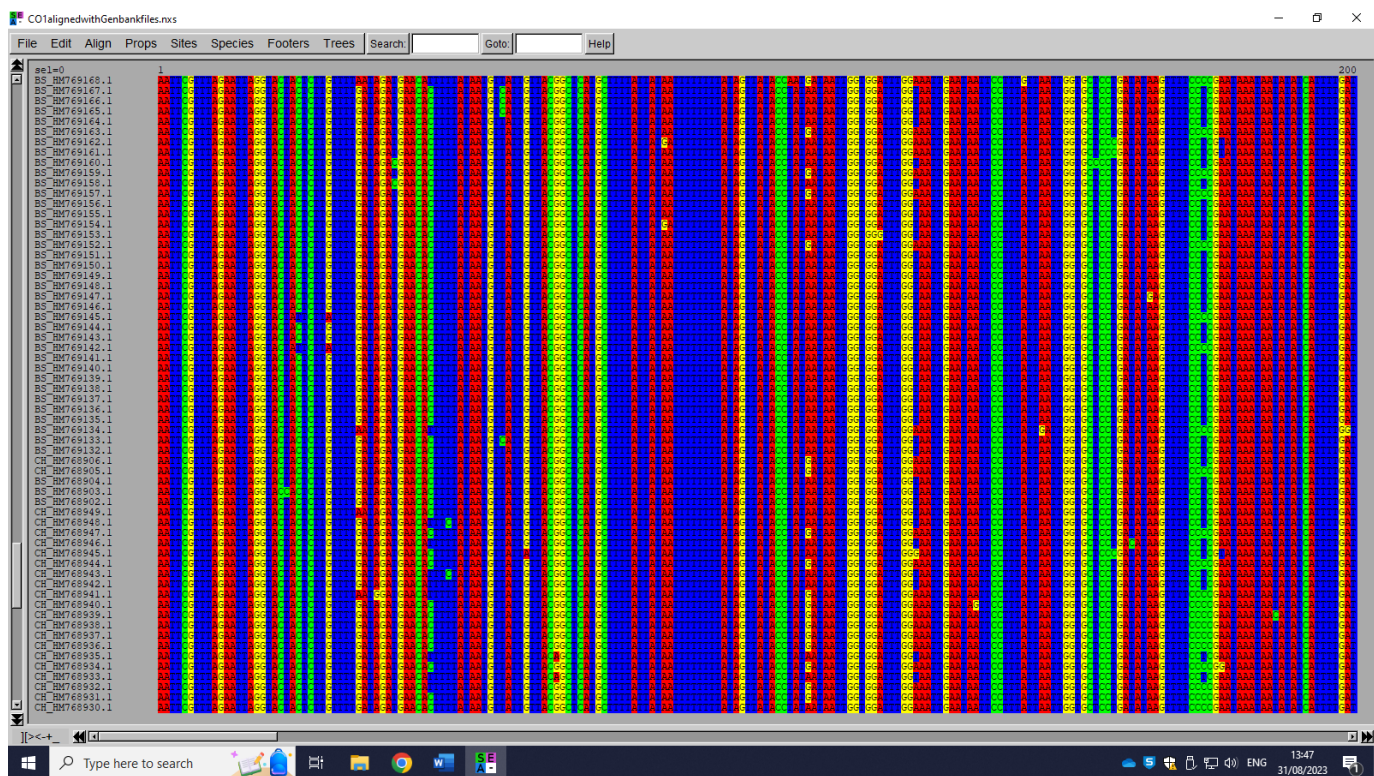
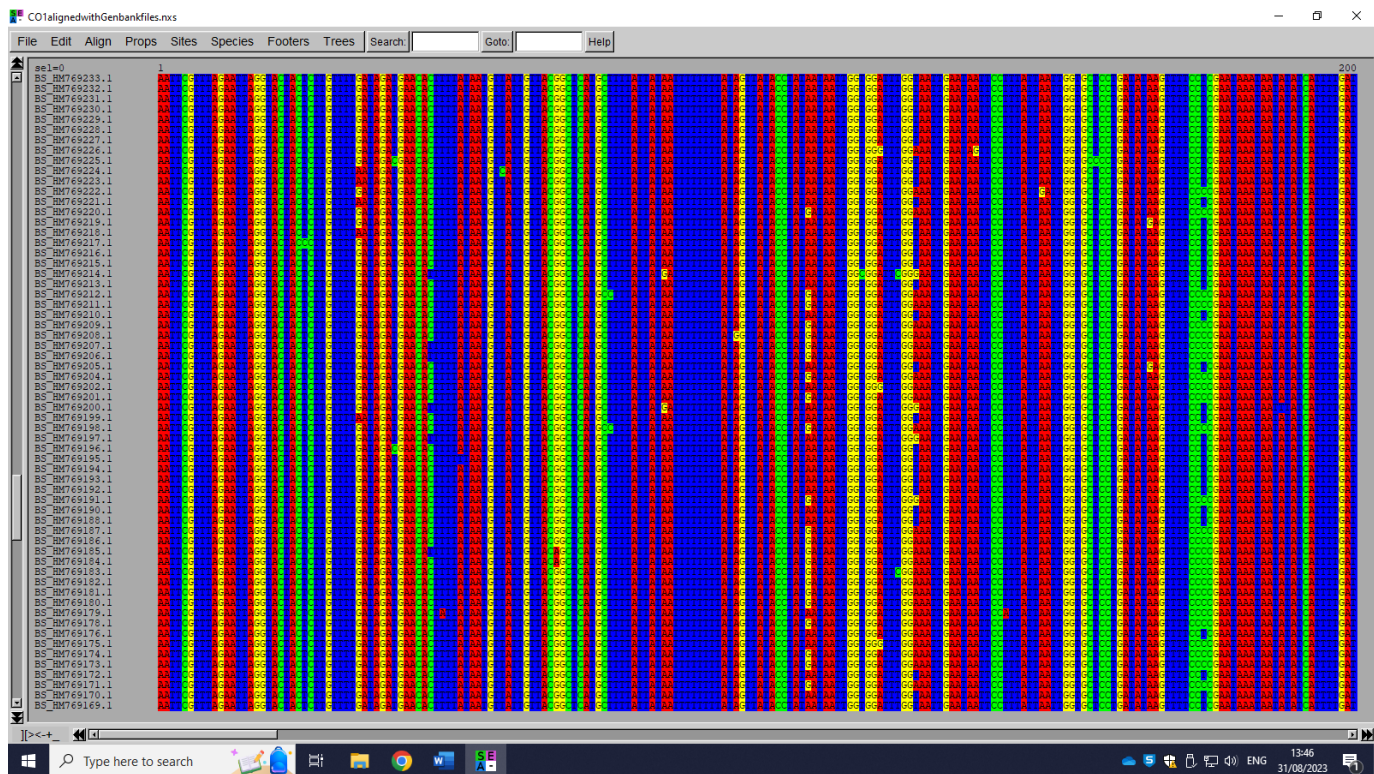
Bujonde shore	(-9.66072, 33.95171)	482m	River	No	30.4	7.2	Sand soil	Y
Matema shore	(-9.49525, 34.02471)	476m	River	No	29.3	6.9	Sand soil	Y
Igalula stream	(-5.63833, 32.62784)	1123m	Stream	No	28.1	7.4	Silt soil	Y
Mwamgongo stream	(-4.62742, 29.65162)	676m	Stream	Yes	25.9	6.8	Silt soil	Y
Bugamba stream	(-4.56683, 29.65203)	767m	Stream	No	32.1	8.1	Silt soil	Y
Kiziba stream	(-4.51933, 29.66061)	916m	Stream	No	29.1	7.2	Silt soil	Y
Babati	(-4.227588, 35.744211)	979m	River	Yes	24.5	8.1	Silt soil	Y
Mbaka	(-9.549804, 33.957001)	479m	River	No	29.5	6.8	Silt soil	Y
Mwaya	(-9.558547, 33.948174)	465m	Stream	No	31.4	7.2	Silt soil	Y
Galana	(-2.201967, 38.058157)	561m	River	No	32.3	7.3	Silt soil	Y
Mpanda	(-6.355559, 31.053331)	1751m	River	No	29.3	7.1	Silt soil	Y
Ibulwa	(-5.215938, 32.669059)	1167m	River	No	31.4	6.8	Silt soil	Y
Shama	(-6.485610, 33.110264)	1136m	River	No	15.3	7.3	Silt soil	Y
Lupa	(-7.904587, 33.303941)	1387m	River	No	13.1	6.7	Sand soil	Y
Mindu dam	(-6.868224, 37.613939)	276m	Dam	No	29.2	7.2	Sand soil	Y
Midiho	(-5.538566, 32.590210)	1173m	River	No	17.3	7.5	Sand soil	Y
Kisisi	(-5.244343, 32.707229)	1196m	River	No	15.3	7.9	Sand soil	Y
Duluti	(-3.385123, 36.786488)	1057m	Dam	No	13.2	9.1	Sand soil	Y
Holili	(-3.378823, 37.620629)	883m	Stream	No	17.3	8.3	Silt soil	Y

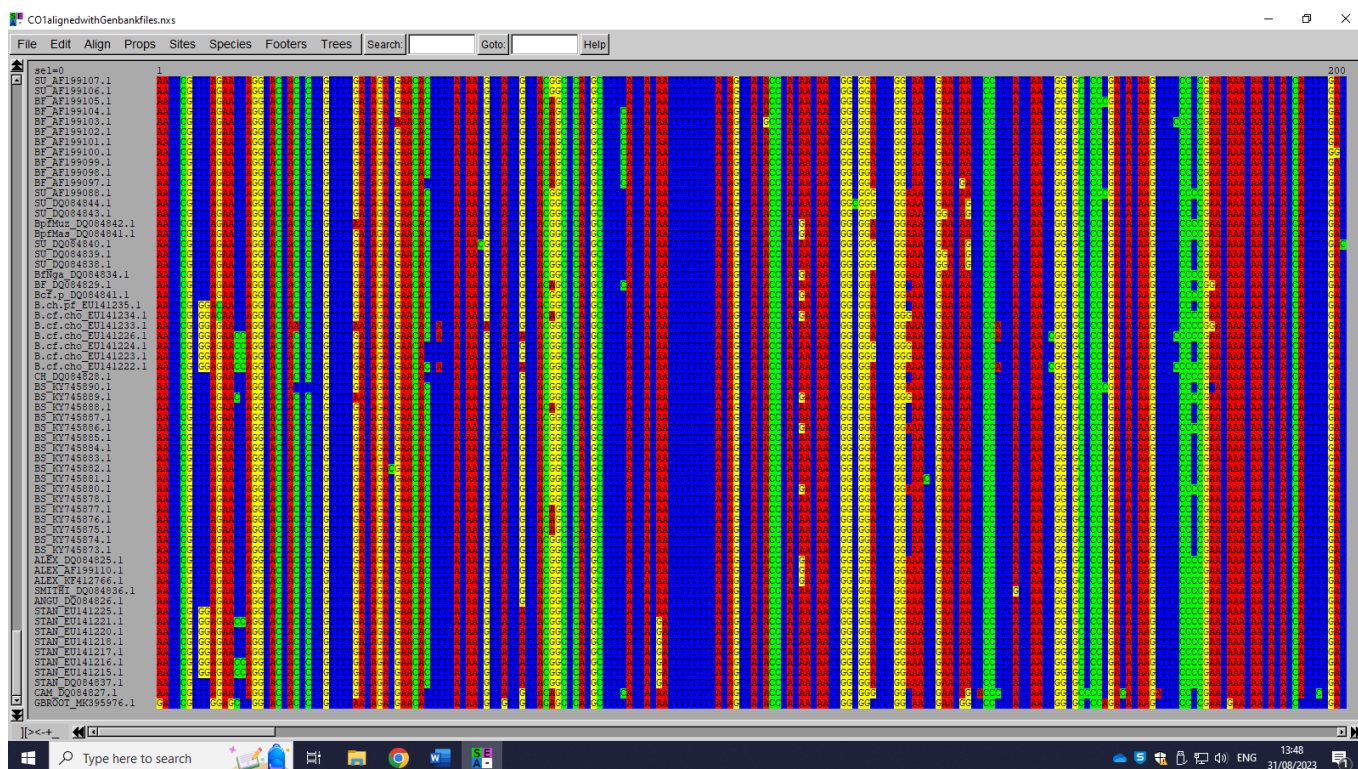
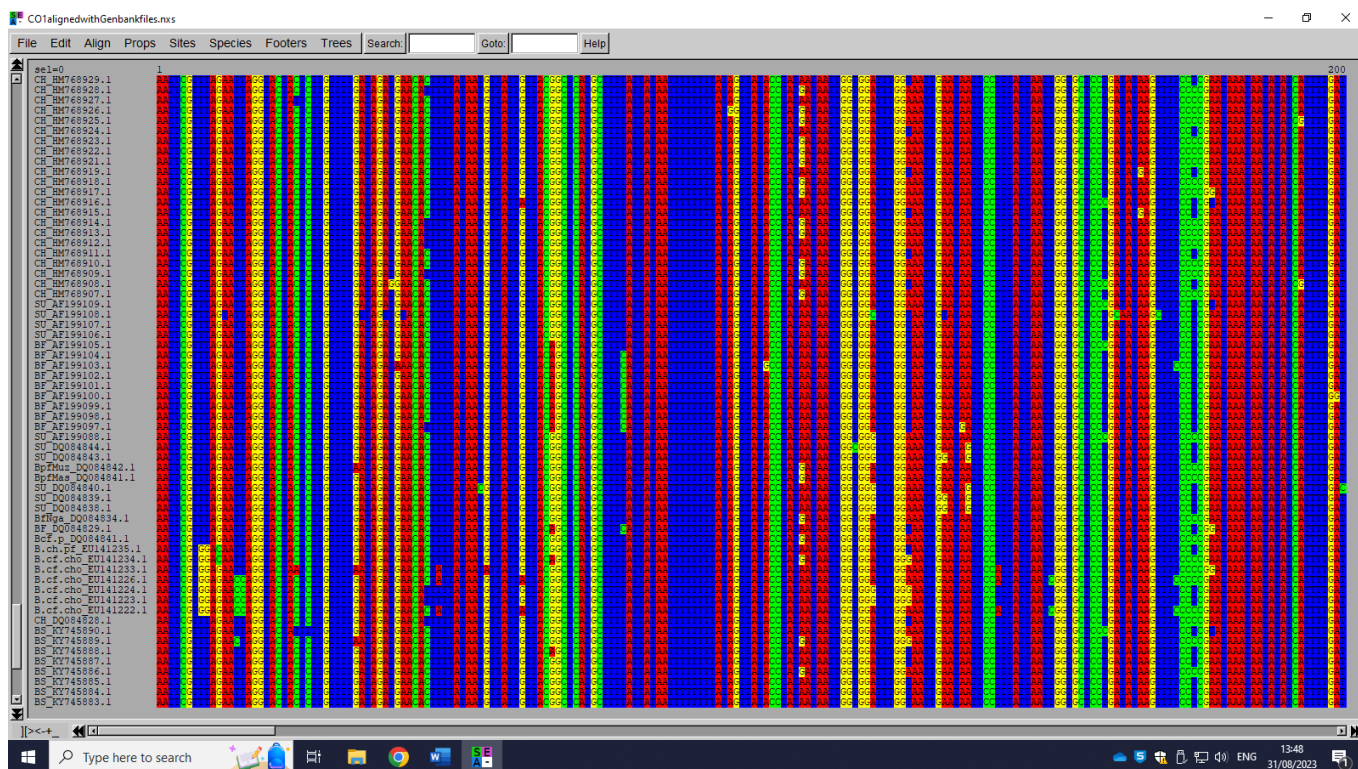
Mkondoa	(-6.825841, 37.161774)	539m	River	No	15.3	6.3	Sand soil	Y
Mkundi	(-6.384851, 37.360174)	412m	River	No	17.6	7.1	Silt soil	Y
Gairo	(-6.127093, 36.867884)	807m	Stream	No	29.7	7.2	Silt soil	Y
Lumuma	(-7.333937, 36.521680)	934m	River	No	29.5	7.3	Sand soil	Y
Bomani	(-1.345125, 34.372426)	1419m	Stream	No	31.2	7.1	Silt soil	Y
Mori	(-1.346410, 34.087468)	1231m	River	No	30.2	6.4	Sand soil	Y
Nkumbu	(-3.695077, 33.460108)	1183m	River	No	31.2	7.9	Silt soil	Y
Mori	(-3.695077, 33.460108)	1442m	River	No	29.4	8.1	Silt soil	Y
Nkaiti	(-3.791196, 35.9164310)	1001m	River	No	31.2	9.2	Sand soil	Y
Pangani	(-3.382294, 37.324104)	834m	River	No	30.8	7.4	Sand soil	Y
Nyankanga	(-1.588600, 33.901086)	1224m	Stream	No	29.2	7.2	Sand soil	Y

Supplementary Image 1: *Biomphalaria* C01 sequences alignment

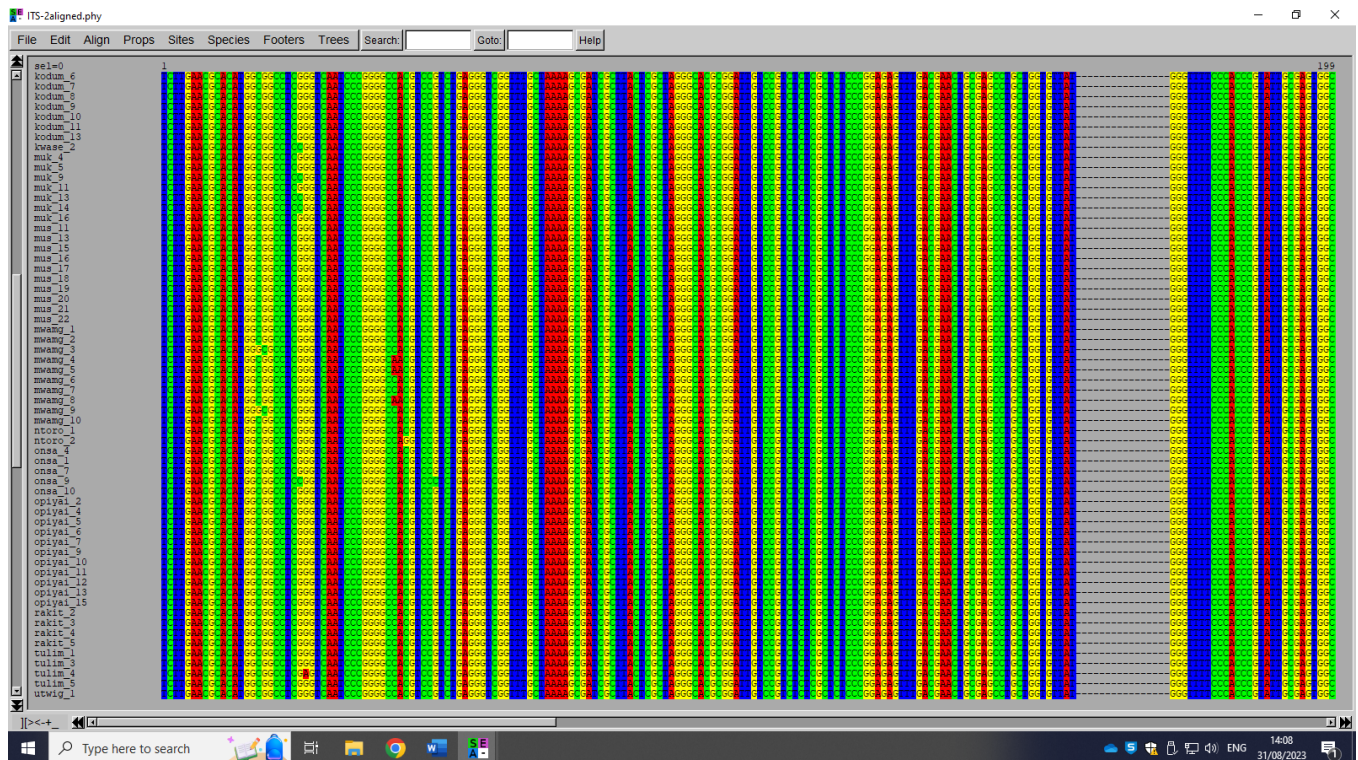
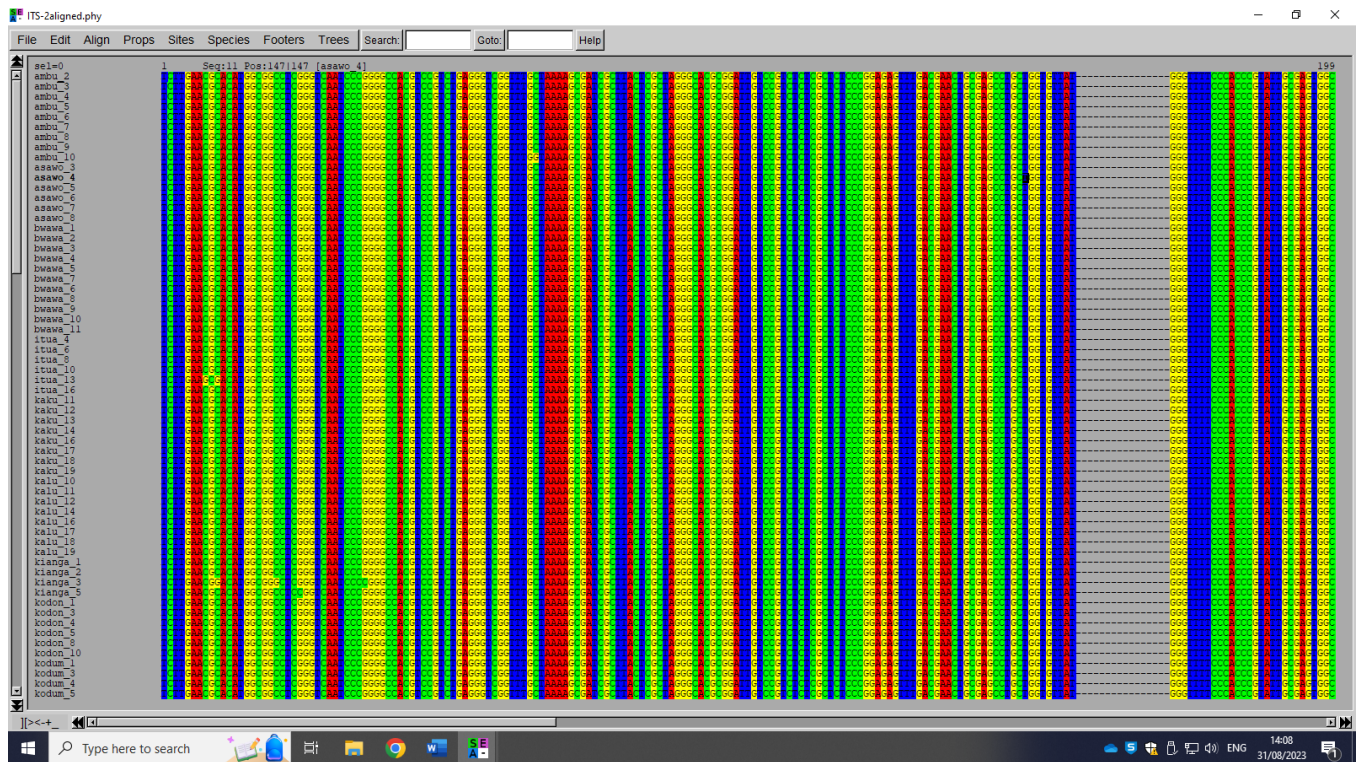


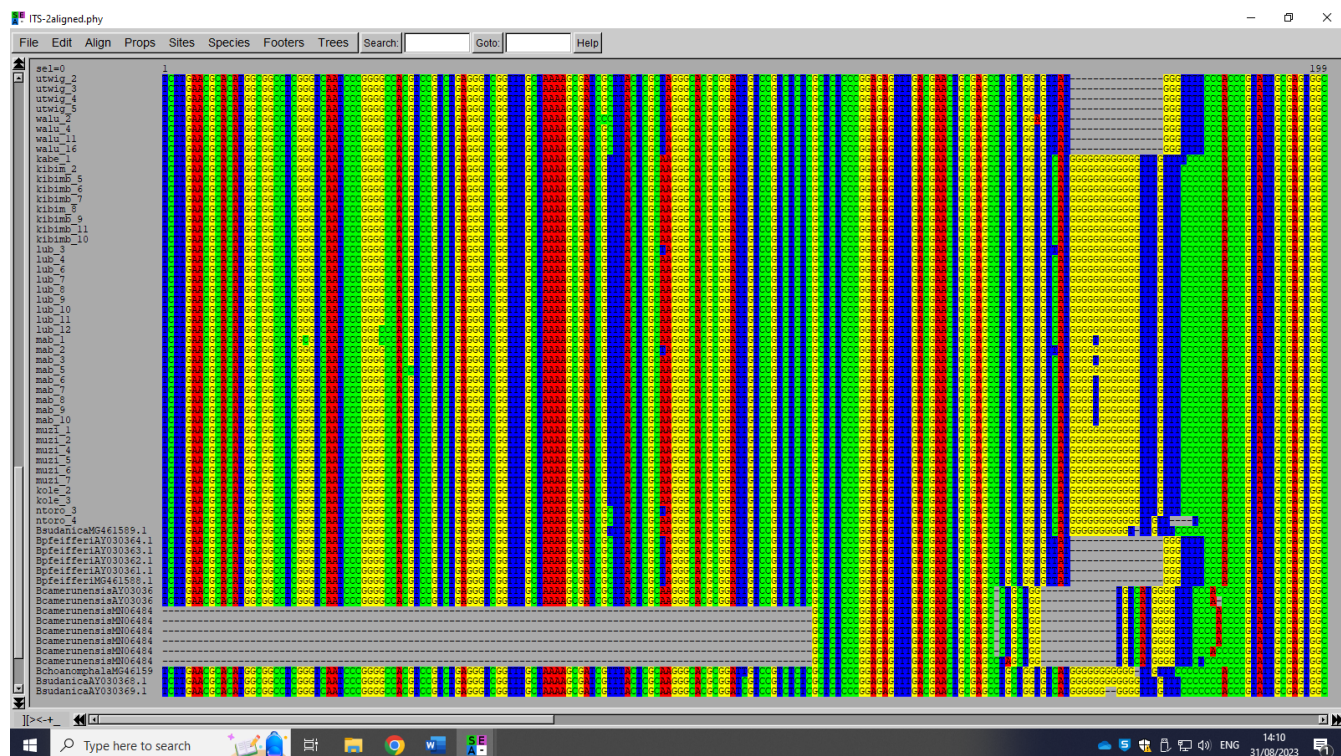




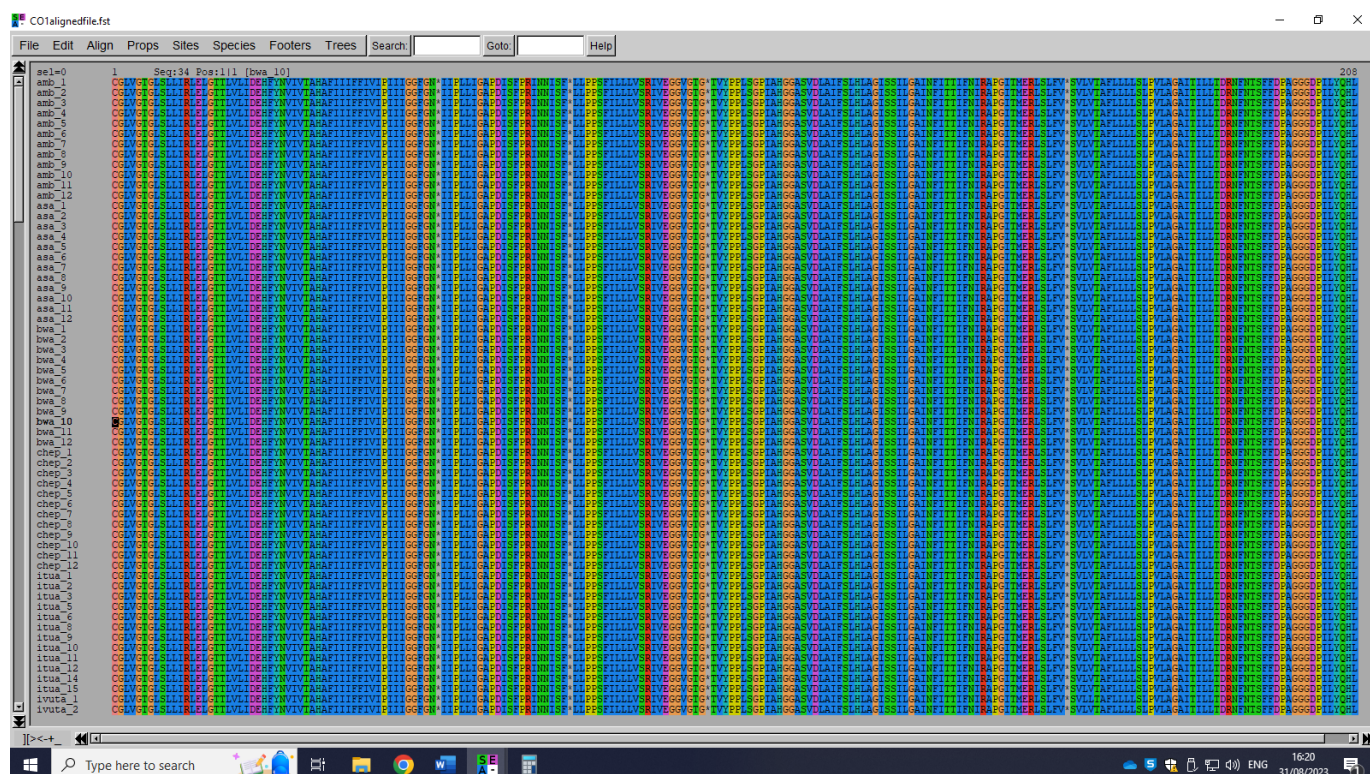


Supplementary Image 2: *Biomphalaria* ITS-2 sequences alignment

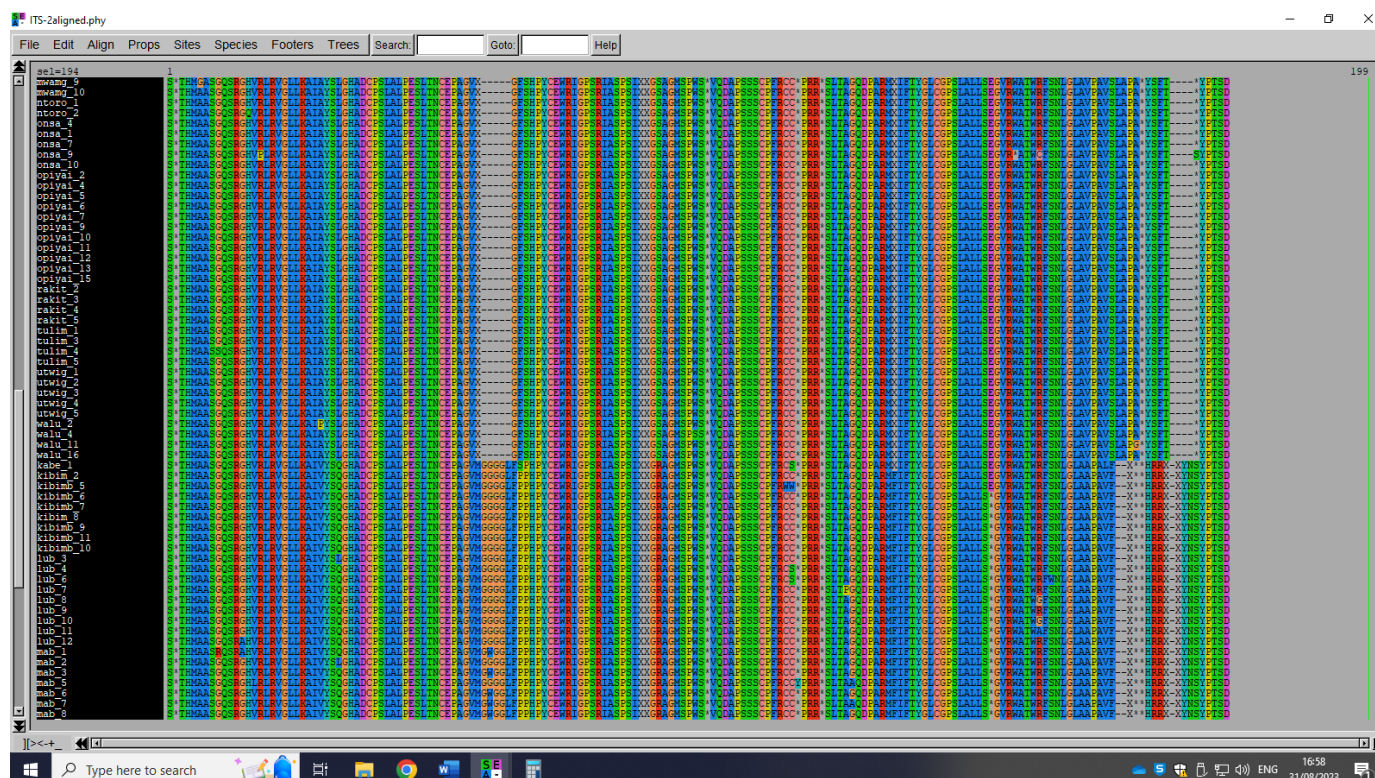




Supplementary Image 3: *Biomphalaria* C01 Amino Acids alignment



Supplementary Image 4: *Biomphalaria* ITS-2 Amino Acids alignment



A Summary of Pairwise Comparisons for C01 sequences

```
Co119Sep2023 Pairwise comparisons - Notepad
File Edit Format View Help
=====
-- Comparisons of pairs of population samples
=====

List of labels for population samples used below:

</data> <pairDistPopLabels time="19/09/23 at 20:21:07">

Label Population name
-----
1: Ambu
2: Asawo
3: Bwawani
4: Chep
5: Itua
6: Ivuta
7: Kakulutuine
8: Kalundu
9: Kiangangi
10: Kodongo
11: Kodumo
12: Kwase
13: Matingani
14: Mukou
15: Musilili
16: Onsando
17: Opiyai
18: Rakite
19: Tulimuimbu
20: Walukuba
21: Mwangongo
22: Utwigu
23: Kole
24: Kibimba
25: Kabere
26: Mabogini
27: Masindi
28: Muzizi
29: Ntoroko
30: Babati
31: Lubiri
</pairDistPopLabels> <data>

Ln 1, Col 1 100% Windows (CRLF) UTF-8 16:50 20/09/2023
```

```
Co119Sep2023 Pairwise comparisons - Notepad
File Edit Format View Help
Population pairwise FSTs
=====

</data> <PairFstMat time="19/09/23 at 20:21:07">

Distance method: Pairwise difference

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
1 0.00000
2 0.00000 0.00000
3 0.89091 0.89091 0.00000
4 0.00000 0.00000 0.89091 0.00000
5 1.00000 1.00000 0.93939 1.00000 0.00000
6 0.94919 0.94919 0.88453 0.94919 0.86155 0.00000
7 1.00000 1.00000 0.91862 1.00000 1.00000 -0.01359 0.00000
8 0.95663 0.95663 0.89811 0.95663 0.00826 0.71076 0.81818 0.00000
9 1.00000 1.00000 0.95041 1.00000 1.00000 0.92566 1.00000 0.85258 0.00000
10 0.00000 0.00000 0.88621 0.00000 1.00000 0.94698 1.00000 0.95455 1.00000 0.00000
11 0.31429 0.31429 0.81562 0.31429 0.93315 0.85652 0.90345 0.88000 0.94760 0.30000 0.00000
12 1.00000 1.00000 0.84416 1.00000 1.00000 -1.00000 0.00000 0.63636 1.00000 1.00000 0.80690 0.00000
13 1.00000 1.00000 0.93939 1.00000 0.00000 0.86155 1.00000 0.00826 1.00000 1.00000 0.93315 1.00000 0.00000
14 1.00000 1.00000 0.91862 1.00000 1.00000 0.92250 1.00000 0.93939 1.00000 1.00000 0.90345 1.00000 1.00000 0.00000
15 1.00000 1.00000 0.91483 1.00000 1.00000 -0.02161 0.00000 0.81010 1.00000 1.00000 0.89879 0.00000 1.00000 0.00000 0.00000
16 0.13636 0.13636 0.80214 0.13636 0.93357 0.87482 0.90983 0.89055 0.94602 0.12522 0.22094 0.82727 0.93357 0.90983 0.90567 0.00000
17 0.00000 0.00000 0.87543 0.00000 1.00000 0.94193 1.00000 0.94970 1.00000 0.00000 0.26822 1.00000 1.00000 1.00000 1.00000 0.10000 0.00000
18 0.00000 0.00000 0.82353 0.00000 1.00000 0.91813 1.00000 0.92561 1.00000 0.00000 0.89055 1.00000 1.00000 1.00000 1.00000 -0.07042 0.00000 0.00000
19 1.00000 1.00000 0.90094 1.00000 1.00000 0.90652 1.00000 0.92513 1.00000 1.00000 0.88154 1.00000 1.00000 1.00000 1.00000 0.89842 1.00000 0.00000
20 0.00000 0.00000 0.86217 0.00000 0.97959 0.92585 0.97172 0.93615 0.98361 -0.00763 0.25092 0.94595 0.97959 0.97172 0.97034 0.09399 -0.02591 -0.18919
21 1.00000 1.00000 0.88107 1.00000 1.00000 0.96601 1.00000 0.96814 1.00000 1.00000 0.93875 1.00000 1.00000 1.00000 1.00000 0.92732 1.00000 1.00000
22 1.00000 1.00000 0.85455 1.00000 1.00000 0.96498 1.00000 0.96559 1.00000 1.00000 0.93747 1.00000 1.00000 1.00000 1.00000 0.92691 1.00000 1.00000
23 0.97426 0.97426 0.95795 0.97426 0.97426 0.96889 0.97300 0.96296 0.97504 0.97215 0.95946 0.84314 0.97426 0.97300 0.97060 0.95955 0.96672 0.91919
24 0.99730 0.99730 0.98783 0.99730 0.99730 0.99245 0.99725 0.99155 0.99738 0.99717 0.98936 0.99451 0.99730 0.99725 0.99711 0.98803 0.99685 0.99528
25 1.00000 1.00000 0.98322 1.00000 1.00000 0.99123 1.00000 0.98936 1.00000 1.00000 0.98519 1.00000 1.00000 1.00000 1.00000 0.98323 1.00000 1.00000
26 1.00000 1.00000 0.99048 1.00000 1.00000 0.99483 1.00000 0.99423 1.00000 1.00000 0.99197 1.00000 1.00000 1.00000 1.00000 0.99051 1.00000 1.00000
27 0.99006 0.99006 0.97725 0.99006 0.99006 0.98436 0.98959 0.98164 0.99033 0.98930 0.97868 0.95526 0.99006 0.98959 0.98873 0.97774 0.98737 0.97248
28 1.00000 1.00000 0.98948 1.00000 1.00000 0.99432 1.00000 0.99355 1.00000 1.00000 0.99102 1.00000 1.00000 1.00000 1.00000 0.98948 1.00000 1.00000
29 0.73171 0.73171 0.70338 0.73171 0.74180 0.74811 0.72988 0.71752 0.75339 0.71689 0.70567 0.17600 0.74180 0.72988 0.71381 0.71705 0.68149 0.46834
30 1.00000 1.00000 0.99105 1.00000 1.00000 0.99512 1.00000 0.99456 1.00000 1.00000 0.99243 1.00000 1.00000 1.00000 1.00000 0.99104 1.00000 1.00000
31 0.79191 0.79191 0.77724 0.79191 0.79060 0.88060 0.78456 0.77601 0.79510 0.78124 0.77637 0.45594 0.79060 0.78586 0.77284 0.78554 0.75611 0.61627

</PairFstMat> <data>

Ln 1, Col 1 100% Windows (CRLF) UTF-8 16:52 20/09/2023
```

```
Co119Sep2023 Pairwise comparisons - Notepad
File Edit Format View Help

18      19      20      21      22      23      24      25      26      27      28      29      30      31

0.00000
1.00000 0.00000
-0.18919 0.96524 0.00000
1.00000 1.00000 0.97759 0.00000
1.00000 1.00000 0.97756 1.00000 0.00000
0.91919 0.95989 0.96994 0.96968 0.95433 0.00000
0.99528 0.99657 0.99460 0.99702 0.99634 0.89980 0.00000
1.00000 1.00000 0.99511 1.00000 1.00000 0.50000 0.95556 0.00000
1.00000 1.00000 0.99723 1.00000 1.00000 0.89590 0.96908 1.00000 0.00000
0.97248 0.98501 0.98646 0.98842 0.98316 0.37124 0.95366 0.85507 0.95923 0.00000
1.00000 1.00000 0.99693 1.00000 1.00000 0.58824 0.99062 1.00000 1.00000 0.80309 0.00000
0.46834 0.65066 0.72723 0.71120 0.63432 0.10290 0.65643 0.03738 0.63879 0.33272 0.44277 0.00000
1.00000 1.00000 0.99739 1.00000 1.00000 0.91430 0.98122 1.00000 1.00000 0.96590 1.00000 0.67574 0.00000
0.61627 0.72952 0.78986 0.76938 0.71003 0.08309 0.55769 -0.04029 0.52187 0.08556 0.24868 0.23568 0.56070 0.00000

Ln 1, Col 1 100% Windows (CRLF) UTF-8 16:52 20/09/2023
```

A Summary of Pairwise Comparisons for ITS-2 sequences

```
ITS-25ep1923 Pairwise Comparisons - Notepad
File Edit Format View Help
== Comparisons of pairs of population samples
=====

List of labels for population samples used below:
-----
</data> <pairDistPopLabels time="19/09/23 at 21:12:03">

Label  Population name
-----
1:    Ambururu
2:    Asawo
3:    Bwawani
4:    Itua
5:    Kakulutuine
6:    Kalundu
7:    Kiangangi
8:    Kodongo
9:    Kodumo
10:   Kwase
11:   Mukou
12:   Musilili
13:   Onsando
14:   Opiyai
15:   Rakite
16:   Tulimuimbu
17:   Utwigu
18:   Walukuba
19:   Ntoroko
20:   Kabere
21:   Kibimba
22:   Lubiri
23:   Mabogini
24:   Muzizi
25:   Kole
</pairDistPopLabels> </data>

-----
Population pairwise FSTs
-----

</data> <PairFstMat time="19/09/23 at 21:12:03">

Ln 1, Col 1 100% Windows (CRLF) UTF-8 17:05 20/09/2023
```

```
ITS-25Sep1923 Pairwise Comparisons - Notepad
File Edit Format View Help
25: Kole
</pairDistPopLabels> <data>

-----
Population pairwise FSTs
-----

</data> <PairFstMat time="19/09/23 at 21:12:03">

Distance method: Pairwise difference
1      1      2      3      4      5      6      7      8      9      10     11     12     13     14     15     16     17     18
1 0.00000
2 -0.10565 0.00000
3 -0.01220 -0.11618 0.00000
4 0.03522 -0.00000 0.06780 0.00000
5 0.05421 0.03170 0.09135 0.03749 0.00000
6 0.07986 0.04278 0.12697 0.04390 0.07725 0.00000
7 0.25193 0.19791 0.35488 0.14489 0.22736 0.08623 0.00000
8 0.00552 -0.00000 -0.06452 0.00000 0.02652 0.04208 0.21715 0.00000
9 0.04873 -0.00585 0.04444 0.01641 0.05075 0.02339 0.15903 -0.02159 0.00000
10 0.42308 0.71429 0.83333 0.33333 0.38095 -0.12925 -0.55556 1.00000 0.20000 0.00000
11 0.18729 0.23179 0.31900 0.16227 0.18654 0.12377 0.19828 0.30290 0.12703 0.00000 0.00000
12 0.03945 -0.01923 0.00524 0.01887 0.05113 0.05725 0.24178 -0.05882 -0.04129 0.53846 0.19459 0.00000
13 0.01045 -0.02321 0.10262 0.01573 0.06150 -0.02995 -0.01803 0.04000 -0.03055 -0.33333 0.02170 0.01445 0.00000
14 0.09007 0.10811 0.00000 0.10811 0.12068 0.13858 0.38227 0.00000 0.05000 1.00000 0.42537 0.01000 0.16981 0.00000
15 -0.06195 -0.08108 -0.12532 -0.08108 -0.04523 -0.03026 -0.01803 0.00000 -0.08360 1.00000 0.22222 -0.12150 -0.05263 0.00000 0.00000
16 0.00339 0.01935 0.09648 -0.02970 0.00775 -0.01208 0.09524 0.11111 -0.03439 0.60000 0.20198 -0.01433 -0.03545 0.27072 0.00000 0.00000
17 0.00994 0.00339 0.04963 -0.01538 0.01878 0.01834 0.15187 0.04000 -0.01945 0.66667 0.21469 -0.02041 0.00000 0.16981 -0.05263 0.00641 0.00000
18 0.09163 0.07538 0.20571 0.03297 0.08046 0.01666 0.07843 0.11111 0.05535 0.00000 0.17867 0.10693 -0.00515 0.27072 0.00000 0.00000 0.04040 0.00000
19 0.48147 0.38822 0.54626 0.38489 0.45732 0.42838 0.26496 0.39655 0.52071 -0.37654 0.43264 0.51605 0.32183 0.55276 0.28526 0.28254 0.34385 0.27469
20 0.98366 0.99344 0.99643 0.98058 0.98197 0.94367 0.89247 1.00000 0.97324 1.00000 0.98889 0.98830 0.95367 1.00000 1.00000 0.99024 0.99219 0.96154
21 0.97323 0.97471 0.98192 0.96962 0.97185 0.95306 0.94172 0.97753 0.96849 0.96213 0.97426 0.97658 0.95889 0.98406 0.97311 0.97028 0.97276 0.96192
22 0.96569 0.96533 0.97468 0.96078 0.96400 0.94660 0.93424 0.96797 0.96209 0.94872 0.96568 0.96930 0.95020 0.97671 0.96227 0.95976 0.96287 0.95231
23 0.97081 0.97170 0.97932 0.96701 0.96941 0.95179 0.94116 0.97434 0.96658 0.95896 0.97152 0.97414 0.95690 0.98136 0.96976 0.96716 0.96970 0.95957
24 0.95798 0.95513 0.96963 0.94937 0.95526 0.93419 0.91147 0.95847 0.95488 0.91850 0.95652 0.96287 0.93450 0.97202 0.94842 0.94507 0.95077 0.93518
25 0.96206 0.95929 0.97733 0.94931 0.95821 0.92624 0.87337 0.96472 0.95690 0.79817 0.96061 0.96842 0.92266 0.98057 0.94759 0.94078 0.95193 0.92079

</PairFstMat> <data>

-----
```

```
ITS-25Sep1923 Pairwise Comparisons - Notepad
File Edit Format View Help

15      16      17      18      19      20      21      22      23      24      25

30 0.00000
33 0.00641 0.00000
36 0.00000 0.04040 0.00000
39 0.28254 0.34385 0.27469 0.00000
42 0.99024 0.99219 0.96154 -0.17060 0.00000
45 0.97028 0.97276 0.96192 0.51131 0.65079 0.00000
48 0.95976 0.96287 0.95231 0.51954 0.55556 0.13335 0.00000
51 0.96716 0.96970 0.95957 0.52671 0.65455 0.17965 0.03731 0.00000
54 0.94507 0.95077 0.93518 0.42305 0.36585 0.13136 0.02560 0.02786 0.00000
57 0.94078 0.95193 0.92079 0.11111 -0.15789 0.39840 0.33384 0.38938 0.15029 0.00000
```

